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# The Effects of Cortisone on the Mitotic Activity and Histology of the Incisor in the Magnesium Deficient Rat

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THE EFFECT OF CORTISONE ON THE MITOTIC ACTIVITY  
AND HISTOLOGY OF THE INCISOR IN THE  
MAGNESIUM DEFICIENT RAT

by

Edward Stanley Salkin

A THESIS

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## LIFE

Edward Stanley Salkin was born on July 8, 1932, in Los Angeles, California.

He was graduated from Dorsey High School, Los Angeles, California, in June, 1950. He entered the University of California at Los Angeles in September, 1950 where, in June, 1953, he received the degree of Associate in Arts. He entered University of Pacific School of Dentistry, San Francisco, California, in September, 1953 and graduated in June, 1957 with the degree of Doctor of Dental Surgery.

In June, 1957 he enlisted in the United States Army and served two years in Frankfurt, Germany in the Dental Corps, then returned to Fort Hamilton, New York where he was honorably discharged with the rank of Captain in June, 1959.

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## I. INTRODUCTION

Magnesium is essential for the maintenance of homeostasis in the biological systems of plants and animals. Willstatter (1903) demonstrated that the magnesium ion occupies the central portion of the chlorophyll molecule. Photosynthesis is dependent upon the integrity of the chlorophyll molecule for the conversion of solar energy into chemical energy in the form of adenosine triphosphate (ATP). With the removal of the magnesium ion from the chlorophyll molecule, photosynthesis does not occur; when it is replaced this process proceeds normally.

It is known that all enzyme reactions in animal cells catalyzed by ATPase require the presence of magnesium ion. In addition, magnesium may serve as an intermediate complexing agent that is responsible for the unwinding of the parent DNA chain (Aikawa, 1971). Mazia (1954) felt that changes in magnesium ion concentration could be a factor determining chromosomal aberration, since it is known that sections of the chromosomes are bound by calcium and magnesium. Magnesium is essential for protein synthesis. It activates numerous enzymes involved in this process, it is necessary for the coupling of M-RNA to the ribosome and maintenance of ribosomal structure is dependent on magnesium (Wacker, 1969).

A deficiency of this mineral in rats produces a number of histopathological changes in a variety of tissues and organs. In addition, grossly demonstrable changes such as hyperemia of the skin, loss of hair, tachycardia and convulsions develop. The magnitude of these changes is proportional to the level of the deficiency, the length of time in progress and the age of the animals. In young animals the onset of the deficiency occurs

sooner and is generally more severe than in mature animals. Since magnesium is readily available in soil and water, a dietary deficiency is rarely observed. Deficiencies due to malabsorption, excessive loss, or deficiencies in enzyme systems activated by magnesium are more commonly seen.

Magnesium deficiency severely interferes with the metabolism of both developing and mature oral tissues, resulting in serious histopathological and gross changes. Numerous reports have shown that the rat incisor and supporting structures are very much dependent on adequate amounts of magnesium for normal maintenance and growth (Becks and Furuta, 1939, 1941, 1942; Bernick and Hungerford, 1965; Duckworth and Godden, 1940; Gagnon, Schour and Patras, 1942; Irving, 1940; Klein, Orent and Mc Collum, 1935; Trowbridge and Seltzer, 1967; Trowbridge and Jenks, 1968; Trowbridge, Louchard and Jenks, 1971; Watchorn and Mc Cance, 1937).

The rate of incisor eruption is also greatly inhibited as a result of magnesium deficiency (Duckworth and Godden, 1940; Gagnon, Schour and Patras, 1942; Kiely, 1967). Cortisone is also known to accelerate incisor eruption in normal animals. Removal of various endocrine glands causes a decrease in this rate while replacement with cortisone restores the eruption to normal values (Domm and Marzano, 1954; Domm and Leroy, 1955; Garren, 1955; Garren and Greep, 1960; Goldsmith and Ross, 1956; Kiely, 1967; Leroy and Domm, 1955; Parmer, Katonah and Angrist, 1951). However, the manner in which this steroid stimulates eruption is still unknown. Therefore, since a diet deficient in magnesium has been found to cause severe disturbances in the growth of incisor tissues and to retard eruption and since cortisone has been shown to somewhat increase eruption even in these deprived rats (Kiely, 1967), it is felt that a study to determine the effect of this cortical steroid on mitotic activity in the magnesium deficient incisor would be in order.



## II. REVIEW OF LITERATURE

### A. Magnesium Deficiency in Animals

#### 1. General Tissue Effects

Kruse, Orent and Mc Collum (1932) found that magnesium deficiency in rats led to vasodilatation, hyperirritability, cardiac arrhythmia, spasticity and tonic-clonic convulsions followed by death.

Watchorn and Mc Cance (1937) studied subacute dietary magnesium deficiency in young rats. After ten days on the diet, hyperemia of the skin, hair loss and some nervousness were noted. These symptoms passed in seven to ten days. After twelve weeks, appetite loss, poor appearance, calcified kidneys, and brittle bones were in evidence. The blood level of magnesium fell to one half normal after fourteen days on the diet with no further decrease noted. Bone magnesium decreased to two-thirds the normal level. The phosphatase content of blood, bone and kidney was normal. An increase in the percentage of water was evident in the kidney, liver and skin, but only the kidney showed a statistically significant difference. Diarrhea and melana were seen by the tenth day of the deficiency. The feces were black and tarry and displayed the presence of blood. Loss of weight was also observed. With the onset of hyperemia, the general condition of the animal improved. The diarrhea ceased, the appetite improved and irritability disappeared. A sticky red exudate on the tail, paws and ears was accompanied by an aromatic odor. There was a decrease in abdominal and subcutaneous fat. Some male rats showed small areas of hemorrhage in the wall of the bladder and stomach. Calcareous deposits were seen in the cortex and medulla of the kidney, but nephritis and abnormal glomerular changes were absent. The liver appeared

normal histologically. The skin showed areas of focal necrosis, ulceration and acute inflammation. The tail revealed excessive keratinization and desquamation of the outer layers. Soft tissue magnesium was less than normal but not significantly so. The kidney showed an increase in the concentration of calcium. However, sodium and potassium metabolism was not affected by the deficiency.

Tufts and Greenberg (1937) studied magnesium deficiency in rats weighing between 40 and 100 gm. They observed two phases of the deficiency. In the first phase vasodilatation, hyperemia and hyperexcitability were evident. The level of plasma magnesium was seen to decrease initially and then increase to peak values after the onset of hyperexcitability. The magnesium content of erythrocytes was reduced to one-half normal. Growth proceeded at a normal rate, but an overall reduction in body magnesium was seen. Phase two revealed malnutrition, cachexia and kidney damage. The plasma level of magnesium fell to one mg%. The amount of calcium was seen to increase by 50 to 100% in cardiac and skeletal muscle, while a fifteen fold increase was noted in the kidney. The water content of bone and kidney also showed an increase. The magnesium content of bone was seen to be two-thirds that of normal, correlating with the findings of Watchorn and Mc Cance (1937). Five mg% of magnesium was found to be the minimum necessary for normal growth, unless large amounts of calcium were added to the diet. Addition of calcium caused a more severe magnesium deficiency so that more magnesium was needed in order to counteract this effect.

Duckworth and Godden (1940) investigated magnesium deficiency in the rat and found that bone acted as a reservoir yielding magnesium as needed to other body tissues.

Gagnon, Schour and Patras (1942) noted a slight reduction in the weight of rats fed a magnesium deficient diet.

Aikawa, Gordon and Rhoades (1960) followed the distribution of  $^{28}\text{Mg}$  in nine normal and 16 diseased patients. Within a few hours after injection the

normal patients showed a rapid disappearance of plasma  $^{28}\text{Mg}$ . On the average, 20% of the isotope was found in the urine after 24 hours. Fecal loss was negligible and equilibration occurred in the bile within 18 hours. The specific activity of the plasma and urine stabilized within 18 hours and showed a gradual decrease thereafter. Exchangeable magnesium averaged 16% of the estimated total body content of magnesium.  $^{28}\text{Mg}$  exchanged slowly with  $\text{Mg}^{++}$  in bone, muscle and erythrocytes. The patients with diabetes mellitus and hepatic disease had similar findings.

Aikawa (1959) studied gastrointestinal absorption of  $^{28}\text{Mg}$  in rabbits and found low renal excretion due to poor gastrointestinal absorption. Absorption was not observed to occur from the large intestine.

Bunce, Chiemchaisri and Phillips (1962) studied the magnesium deficiency syndrome in weanling dogs. The signs of the deficiency took much longer to appear in the mature dog as compared to the pup. Dietary increases of calcium ranging from 0.6 to 0.9% and phosphorous from 0.4 to 0.9% heightened the severity of the deficiency. The effect of phosphorous was more pronounced. Reduction of phosphorous to 0.22% alleviated the signs of the deficiency, but the lowering of calcium to 0.3% had no effect.

Supplements of vitamin C, menadione, inorganic sulfate, or sulfur had no effect on the deficiency. Addition of 250 ppm fluoride to the magnesium deficient diet restricted food intake and growth, and prevented the occurrence of aortic lesions and the accumulation of calcareous deposits, but did not prevent the occurrence of muscular weakness and convulsions or the lowering of serum magnesium.

Aikawa (1963) stated that four-fifths of the body magnesium is found in bone and muscle; the remainder in the soft tissue, with 1% found in the extracellular fluid.

Martindale and Heaton (1964) observed a loss of weight in the deficient adult rats. Femur growth and the plasma level of magnesium were directly correlated; that is, initially both dropped rapidly and then later more slowly.

The heart, brain, kidney, thigh muscle and liver showed reduced levels of magnesium. Calcification first took place in the kidney, then in the heart, liver, skeletal muscle and femur. Secondary hypercalcemia was also seen. Potassium depletion occurred in skeletal muscle, liver and heart. The amount of skeletal magnesium utilized during the deficiency was apparently greater in the post-natal as compared to the adult rat. It was concluded that a normal intracellular concentration of magnesium is apparently necessary for the homeostasis of soft tissue.

Bernick and Hungerford (1965) found an overall decrease in the growth of magnesium deficient rats. Interference in the calcification of cartilage and bone was noted. The hypertrophied zone of the posterior head of the tibia was widened and the proliferating zone of cartilage was narrowed. This, coupled with strong staining reactions (deep red with periodic acid-Schiff and intense violet with toluidine blue) indicated an interference in cartilage calcification. It is thought that the deficiency reduces enzyme activity which is necessary for the maintenance and growth of tissues.

Trowbridge and Seltzer (1967) investigated the rat under the influence of magnesium deficiency and found an interference with collagen formation and sulfation of protein polysaccharides in the organic matrix of bone. Magnesium deficiency suppressed enzyme systems which ultimately affected alkaline phosphatase activity. Classical signs of decreased body growth, hyperemia of the ear, hyperexcitability, and kidney calcification were seen.

Utilizing  $^{28}\text{Mg}$  Aikawa and David (1969) studied the effects of a magnesium deficient diet on rabbits. When the intake of magnesium was 0.3 mEq/day, the renal excretion of magnesium fell below 0.8 mEq/day. After one week the serum magnesium level fell to 1.2 mEq/l where it remained. After one month hair loss and the lack of hair luster were evident. The animals did not become hyperirritable. The magnesium content was shown to decrease in the lung and bone. In a second experiment the diet contained 0.8 mEq/l of magnesium. The serum level fell 43% after one week from 1.93 mEq/l to 1.09 then

to 0.98. Hyperexcitability occurred after one week. In addition, tachycardia, vasodilatation and hair loss were seen in the second and third week. From the fourth to sixth week the rabbits stopped eating. Signs of malnutrition, renal failure, and jaundice appeared and became quite severe. The average weight at sacrifice was 40% lower than in controls. Tissue analyses demonstrated that the magnesium content was less than normal in the appendix, bone cortex, and large intestine. No change was seen in skeletal muscle, skin, testis and neck lymphoid tissues.

## 2. Oral Tissue Effects

Klein, Orent and Mc Collum (1935) in their study on the effects of magnesium deficiency on the teeth and supporting tissues in rats, reported the following gross changes: the oral mucous membrane was blanched, the gingiva was a bulbous mass of smooth whitish gray tissue, the lower incisors were separated by a tissue mass, and the molars, which were mobile, were covered by calculus and were surrounded by a large tissue mass. Histologically, the periodontal ligament exhibited a decrease in cell number and an increase in the amount of pink-staining intercellular substance. There was a deep blue staining amorphous material in the lamina dura. After three months the bone around the molars was replaced with large masses of deeply stained pink tissue containing many spindle-shaped cells. The incisors were surrounded by a similar mass. The dentin of the molars and incisors was striated.

Watchorn and Mc Cance (1937) noted the effects of dietary subacute magnesium deficiency upon the teeth of rats. The incisors were brittle, chalk-white and very mobile. Grossly, the molars appeared normal. Histologically, the incisors showed irregular and striated dentin that stained alternately with hematoxylin and eosin. The odontoblasts were in various stages of degeneration and were absent in many instances. The capillaries showed enlargement. Pulp stones were frequently evident in the molars and these teeth also showed an inhibition in growth. In the deficient teeth the water content showed an increase while the amount of magnesium was reduced to one-half. Tufts

and Greenberg (1937) also noted an increase in the water content and a decrease in magnesium content to one-half in the teeth of magnesium deficient rats.

Becks and Furuta (1939) studied the effect of magnesium deficiency on the enamel epithelium of the rat. The ameloblastic layer was initially distorted and after 186 days, this layer was completely absent from the apical region resulting in connective tissue directly contacting dentin. In the incisal area, amorphous chromatophilic deposits were found in the subepithelial connective tissue and the reduced enamel epithelium was replaced by an acellular substance. Disturbances in enamel formation led to hypoplasia (Becks and Furuta, 1941). Thus, they stated that magnesium is essential for the formation of enamel.

Becks and Furuta (1942) studied dentin and pulpal changes under the influence of magnesium deficiency. Rats were placed on the diet for 41, 84 and 186 days. Histopathologic changes increased in severity with time. These changes were more pronounced in the incisors than in the molars. After 41 days, the normally thick labio-incisal dentin became thin and the lingual dentin was striated. Vacuolization and degeneration of the odontoblasts was observed. After 84 days severe striating and much folding of the labial dentin was evident near the apex. The folds of dentin were interpreted as an indicator of delayed eruption. After 186 days the apical folding had increased and it was present both labially and lingually. The incisor pulp, after 41 days, showed amorphous calcification in the incisal one-third with evidence of some calcification extending to the distal one-half. After 84 days, two-thirds of the pulp was calcified and almost complete calcification had occurred by 186 days. Four types of pulpal calcification were evident in the incisor:

1. An irregular calcified mass bordered by odontoblasts moving toward the pulp center.
2. Amorphous layers of calcified deposits associated with severe striations of the dentin. Calcified spurs formed on the pulp wall.

3. The center pulp tissue showed calcification independent of pre-dentin or irregular dentin deposition. The pulp became more acellular.
4. Deposits of densely calcified, round, black structures in the connective tissue of the pulp were seen. The pulp of the molars showed stellate-shaped cells becoming round with loss of their processes after 186 days. This was the severest pulp change seen in the molars. The odontoblasts were irregular and no longer in contact with the predentin zone.

Duckworth and Godden (1940) investigated magnesium deficiency in the rat and found that even though the incisor was not depleted of magnesium, there was a decrease in the rate of deposition.

Irving (1940) in studying the rat incisor found the calcification rhythm of the predentin, as well as the cells of the enamel organ and odontoblasts to be upset during magnesium deficiency, but this situation was reversed by supplementing the diet with adequate amounts of magnesium. Changes brought about by the deficiency included an increase in width and stratification of the predentin, calcareous granules distorting the enamel organ, followed later by more severe atrophic changes. The dentin was translucent and stained poorly with hematoxylin and eosin. The molars showed only slight changes with some stratification of dentin.

Gagnon, Schour and Patras (1942) noted a widening of the periodontal ligament and a folding of the basal dentin in the magnesium deficient rat incisor.

Bernick and Hungerford (1965) found an interference in dentin formation in the deficient rat incisor. This was evidenced through the use of the toluidine blue and P.A.S. stains. The dentin displayed a wide uncalcified zone, with a thin calcified line separating this zone from the predentin. The odontoblasts were atrophied. Silver nitrate impregnation stained the predentin of control teeth black, whereas in the deficient animals the entire dentin

showed the stain. The authors feel that lack of magnesium inhibits enzymatic activity resulting in alterations in the synthesis of collagen, protein and mucopolysaccharides, thereby interfering with subsequent mineralization.

Trowbridge and Seltzer (1967) noted an interference in collagen formation and in the sulfation of protein polysaccharides in the organic matrix of incisor dentin. Here again it was felt that the magnesium deficiency suppressed enzyme systems necessary for dentin formation. Resorption of alveolar crest bone was observed and there was evidence of arrested appositional bone growth. The periodontal ligament was widened and its cellular structure had become atrophied. The incisal dentin showed striations. Although alkaline phosphatase was present in the odontoblastic layer of the dentin many undifferentiated odontoblasts lacked this activity.

Kiely (1967) studied the effect of a magnesium deficient (10 mg %) diet on rats. The lower incisors were dull and chalky compared to the shiny yellow-orange of control teeth. The upper incisors were chalky with pigmented patches. The incisors were more brittle, displayed chipping at the incisal edge and were softer than controls when scratched with a file. The dentin was stratified, and in severe cases showed folding with encroachment on the pulp. The odontoblasts were disorganized with lack of maturation coronally. Connective tissue had replaced the basal section of the incisor in one rat, with normal histology obliterated. Enamel organ degeneration was seen. In many instances, the ameloblasts, stratum intermedium, and the papillary layer were replaced by connective tissue while there was an amorphous substance present in the enamel space. In less severe cases there was a reduction in the height of the cells of the enamel organ and enamel epithelium.

Trowbridge and Jenks (1968) observed an inhibition of appositional growth in the incisor teeth of magnesium deficient rats. Using tetracycline, 25% decrease in rate of dentin formation was observed over a 3 day period. The predentin showed an increase in width. The decrease in the synthesis of



the organic matrices of the teeth and alveolar bone was postulated as causing the suppression of growth. With tetracycline labeling, it was observed that growth of alveolar bone adjacent to the molars had ceased after fourteen days on the deficient diet.

Trowbridge, Louchard and Jenks (1971) studied magnesium deficiency in normal and parathyroidectomized rats. Magnesium deficiency led to striated dentin. When magnesium was added to the diet, the striations disappeared after four days. Parathyroidectomy had no effect on dentinal striation formation. Each dentinal striation had 3 staining zones:

1. Sudanophilic with enlarged dentinal tubules.
2. Alcian-blue and colloidal-iron-positive which reacts with toluidine blue.
3. Positive periodic acid Schiff.

In a given striation, zone 1 was closest to the pulp, followed by 2 and 3.

In studying extracted human teeth with electron probe microanalysis, Green, Eick, Miller and Leitner (1970) found that the normal magnesium content of the cementum and bone was one half that of dentin. Utilizing the same technic Neiders, Eick, Miller and Leitner (1972) found, in young permanent teeth, that cementum contained the least amount of magnesium, while the hypermineralized zone and tubular portion of dentin possessed the greatest amount.

Shaw and Yen (1972) used the spectrophotometer to study extracted human noncarious teeth and observed magnesium concentrations to increase from the surface enamel to the internal dentin.

Johnson (1972) used the electron microprobe to analyze the incisor of rats given a diet containing an excess (0.2%) of strontium. Strontium was seen to compete with magnesium so that a lesser amount of magnesium was found in the enamel and dentin resulting in a smaller and defective incisor. In the enamel and dentin of strontium-laden teeth parallel gradients of

strontium and magnesium were found. In the normal teeth, however, magnesium distribution increased from the enamel surface into the dentin. These observations suggest that magnesium is vital for the process of mineralization and also for the structural integrity of the incisor.

#### B. Magnesium Deficiency and Incisor Eruption

Duckworth and Godden (1940) observed the incisors of young rats to have a faster eruption rate than mature rats. They observed this rate to be only slightly inhibited with acute magnesium deficiency. However, the magnesium content of these incisors remained unchanged while showing an increase in normal rats. This indicated that the loss of magnesium due to abrasion was equal to its deposition in the deficient incisor and that magnesium is not mobilized from the teeth in cases of severe deficiency.

Gagnon, Schour and Patras (1942) also found that incisor eruption in deficient rats was only one-third the normal rate. These investigators utilized a diet containing 1.8 ppm of magnesium, while the diet of Duckworth and Godden (1940) contained 6 ppm. Factors influencing rate of eruption were considered to be:

1. Deceleration of dentin apposition, the enamel-covered dentin was affected more than the cementum-covered part.
2. Alveolar bone deposition was reduced to one-half normal.
3. The periodontal ligament was widened.

Kiely (1967) noted a 42% decrease in the rate of eruption of deficient animals compared to the normal for a ten week period. Beginning with the first week of observation there was a significant difference in eruption rate between the normal and deficient animal. With each ensuing week the difference became more pronounced, so that the rate of the deficient was 30% lower than the normal by the end of the second week and a maximum decrease of 54% was seen during the eighth week. He felt that his results indicated that the process of cell proliferation was a factor in tooth eruption. He concludes that the

magnesium level, within a normal range, is needed for maintenance of incisor tissues and eruption.

### C. Cortisone and Incisor Eruption Rate

Parmer, Katonah and Angrist (1951) found that cortisone enhanced the incisor eruption rate in newborn rats to the extent that these teeth erupted 2.5 days earlier than those of the controls. Domm and Marzano (1954) noted that cortisone increased the eruption rate of the incisor in both young and adult rats. In the newborn rat, the incisor erupted earlier than normal. The mean for the male upper incisor was 2.58 mm per week and 3.31 mm for the lower. The female weekly rate was 2.49 mm for the upper and 3.11 mm for the lower. A constant ratio was maintained for upper to lower incisor rate in both males and females. In both sexes these rates were greatly increased with cortisone administration and upper to lower incisor rate ratios did not change. Hypophysectomized rats showed decelerated incisor eruption rates in both sexes. These animals showed increased rates when given cortisone, but the upper to lower incisor ratio was strongly disturbed. Growth hormone given to newborn rats had little or no effect on incisor eruption, while cortisone enhanced these rates.

Domm and Leroy (1955) and Leroy and Domm (1955) observed premature eruption of the incisor in the newborn rat when cortisone was given to the pregnant mother, to newborn rats or to the fetus in utero.

Goldsmith and Ross (1956) found that cortisone administered to fetal and postnatal rats resulted in an early development of the lower incisor. The eighteen day old cortisone treated fetus revealed incisors with more highly differentiated ameloblasts and odontoblasts than controls. These cells also contained more RNA. At 20 days, in addition to the above observations, the labial pre dentin was widened, and the overall tooth was larger. The lower incisors erupted 2.9 days sooner than those of controls. At 18 days cortisone caused a greater deposition of glycogen in the stratum intermedium. In the

20-day-old fetus glycogen deposition was increased in the stratum intermedium and the labial odontoblasts showed a PAS positive reaction greater than controls. Also, at this time the stratum intermedium showed a stronger alkaline phosphatase reaction than controls. In addition to the above noted changes the post-natal rat also showed an obliterated labial periodontal ligament - the fibers were densely packed and the ameloblasts were disorganized and degenerating. Lastly, there was increased capillarity and capillary dilatation in the experimental group.

Garren (1955) and Garren and Greep (1960) noted that cortisone administration resulted in an accelerated eruption of the adult rat incisor. Kiely (1967) also observed an increase of incisor eruption in the adult female rat following cortisone injection.

From the preceding review, it is apparent that no disagreement exists regarding the observation that cortisone does indeed enhance the eruption of the rat incisor. The reasons for this effect, however are still to be discovered.

#### D. Effects of Cortisone

##### 1. General Tissue Effects

Parmer, Katonah and Angrist (1951) found body growth to be inhibited in rats treated with cortisone.

In studying the cells of the crypts of Lieberkuhn of partially hepatectomized mice, Roberts, Florey and Joklik (1952) noted that cortisone did not affect cell division rate. Cortisone, in large doses, suppressed mitotic activity in the regenerating liver. DNA and RNA concentrations fell in cortisone treated unoperated livers. This was thought to be due to an increase in fat and glycogen deposition and to a fall in liver protein. Therefore the accompanied weight increase of liver was not due to the restoration of liver tissue. It was concluded that cortisone does not inhibit mitotic activity in all the tissues studied.

Fleming (1953) noted a decrease in the body growth of cortisone

treated guinea pigs.

Glickman, Stone and Chawla (1953) found that 4 week old white mice treated with cortisone were less active, smaller and had more hair loss than controls.

Alt, Robbins and Cooper (1955) found that corticotropin caused a decrease in cellularity and total mitotic activity of the bone marrow, spleen and thymus in the rat. In their view, decreased cellularity can be caused by inhibition of mitosis, cell destruction or an increase in cell maturation.

Sissons and Hadfield (1955) worked with young male rats and rabbits. They found that cortisone administration resulted in:

1. Retardation in vertical growth of long bones, a narrowing of the epiphyseal plates, absence of hypertrophic cartilage cells, cessation of cartilage proliferation, and osteoclastic bone trabecule resorption in the metaphyses.
2. Rabbits displayed a complete arrestment of epiphyseal bone growth. Normal growth recurred in the absence of cortisone therapy.
3. Rats showed less severe bone growth retardation and structural changes in the epiphyseal growth zones.
4. In rats the decrease in bone growth was not always accompanied by an increase in density of the metaphyseal bone.

D'Arcy and Howard (1961) gave cortisone acetate to albino rats for six weeks and found that:

1. Cortisone caused a retardation in body growth. Mean control weight was 273 gm compared to 183 gm for the cortisone group.
2. Adrenal and pituitary glands weights were less than controls without consideration for loss of total body weight.

3. When total body weight loss was considered there was no significant difference in the weight of the pituitary gland between control and treated groups.
4. With weight adjustments, the cortisone treated adrenals still weighed less than those of controls.
5. There was no significant difference between the weights of the liver, spleen, seminal vesicles, and prostate gland when total weights of the two groups were adjusted. The experimental liver and spleen weights were less than those of controls without weight adjustment.

The results were interpreted as indicating that cortisone increases body catabolism in a non-specific way with the exception of its specific action on the adrenals.

Frost and Villaneuva (1961) found that cortisone and related corticoids or ACTH depressed lamellar osteoblastic activity in the human when administered over a two week period.

Brandt and Glaser (1959) found no difference in the rate of plasma disappearance or soft tissue uptake of  $^{28}\text{Mg}$  between normal and adrenalectomized rats given a single injection of cortisol.

Aikawa, Harms and Reardon (1960) studied the effect of cortisone on magnesium metabolism in the rabbit. Daily injections of cortisone acetate caused a significant reduction in serum magnesium concentration after 8 and 14 days. On the eighth day there was an increase in exchangeable magnesium and an increased uptake of  $^{28}\text{Mg}$  in the heart, appendix and muscle. The results indicate that cortisone alters the distribution of magnesium in the body.

Hulth and Olerud (1963) studied the effect of cortisone on growing bone in the rat. The metaphysis was abnormally dense with unresorbed cartilage cells and cartilage matrix. Formation of new bone was not observed. The blood vessels were lumpy and did not extend to the metaphyseal cartilage.

When cortisone therapy was discontinued, the bone returned to a normal condition.

Kidson (1967) investigated the effect of cortisol on the regulation of RNA and protein synthesis in lymphoid tissue and found RNA synthesis to be inhibited. In a matter of seconds or minutes, cortisol altered this synthesis suggesting that the hormonal effect is exerted on the initial events in induction and repression. The experiments were carried out on suspensions of rabbit lymphoid cells and adrenalectomized rat spleen cell suspensions.

Farnell (1966) injected cortisone into magnesium deficient rats and observed hypomagnesemia to persist for 28 hours even though these animals were given a magnesium supplemented diet. When daily injections of cortisone (2.5 mg) were given, convulsive activity was delayed and peripheral vasodilatation was partially or completely eliminated.

Nakamoto and Wilson (1968) found that cortisone (40 mg/kg) administered to young rats resulted in retardation of weight gain, skin damage, hair follicle defects and increased mortality. The erythrocyte count remained normal but the lymphocyte count dropped markedly. Atrophy of the adrenal cortex and the lymphoid tissue of the thymus was apparent. After eight days, there was reduced hematopoietic tissue in the spleen, liver and femur. It was concluded that immunosuppressive doses of cortisone could not safely be used in the very young animal.

Henderson, Fischel and Loeb (1971) administered cortisone to young rats and found rapid and complete inhibition of DNA synthesis in the liver. At the same time, there was a stimulation of liver RNA, protein and glycogen. When cortisone administration was discontinued, DNA synthesis returned to normal.

Davidovitch (1971) conducted a radiographic and autoradiographic study on the effects of cortisone on bone growth in young albino rats. He found that the tibia became sclerotic due to continuous matrix formation and cessation of resorption. There was less weight gain in the cortisone treated rats compared

to controls. The tibia of the experimental animals grew less than in controls. With the cessation of cortisone treatment the bones resumed their normal appearance.

In review, cortisone appears to have a stimulatory or depressing effect depending on the tissue or animal studied. Also the age of the animal seems to influence this effect. Various investigators report conflicting results as to the effect of cortisone on cell growth. Kiely (1967) studied incisors of cortisone treated magnesium deficient rats. He noted their pathology not to be as severe as in non-treated magnesium deficient animals.

## 2. Oral Tissue Effects

Fleming (1953) studied the effect of daily injections of cortisone acetate (2.5 mg) on tooth germs of 20 day old guinea pig embryos transplanted into the anterior eye chamber of guinea pig adults. The results were as follows:

1. Transplants vascularized in 24 hours as compared to four days in the controls.
2. Growth of the tooth germ was initially more rapid than in controls.
3. This growth was surpassed by controls after vascularization occurred.
4. Many transplants resorbed upon discontinuation of cortisone injections. This was due to lymphocytic phagocytosis.
5. Control transplants were observed to make attachment to the iris of the host, while this process did not occur with cortisone treatment.

Glickman, Stone and Chawla (1953) observed the effect of cortisone on the periodontium of white mice. One half male and female litter mates were injected initially at 4 weeks of age, with 0.5 mg of cortisone daily for 14 days. Grossly, the oral tissues appeared similar in the control and experimental



groups. Histologically, the height of interdental alveolar bone was reduced with cortisone treatment, however, when the photographs are examined no difference is apparent. The cortisone group showed minimal osteoblast or osteoclast activity, enlarged osteocytes, an edematous periodontal ligament, fibroblasts that were reduced in number and size, and a reduction in the number of collagen fibers. These fibers had a wavy appearance and were loosely arranged due to intercellular edema.

Applebaum and Seelig (1955) studied histologic changes in the jaws and teeth of young rats treated for one or two months with cortisone. These animals showed alveolar bone loss and pulpal blood vessel dilatation in the molars.

Goldsmith and Ross (1956) studied the histological and histochemical effects of cortisone on the lower incisor of fetal and post natal rats. They found an increase in the alkaline phosphatase and ribonucleic acid content of ameloblasts and odontoblasts, as well as increased glycogen content in the stratum intermedium of the fetal rats. In the cortisone treated postnatal rats they observed premature differentiation of the incisor. In the older postnatal rats an overgrowth of alveolar bone, degeneration of ameloblasts and disorganization of the periodontal ligament was observed.

Blackey and Johannessen (1964) found that the jaws of immature albino rats were reduced in size after receiving 0.2 mg cortisone/gm of food for 20 days followed by a normal diet for 20 days.

In another experiment, (Johannessen 1964), weanling rats received 0.2 mg cortisone/gm of food for 20 days. One group received a total of 20 gm of food, a second group, 40 gm and a third control group received food ad libitum. Cortisone was observed to inhibit apposition of dentin, especially on the occlusal surface of the pulp chamber. An inhibition of body growth and food intake was seen in the cortisone treated rats.

Anneroth and Bloom (1966) observed that cortisone treatment (50 mg/day) resulted in a disorganization, increase in cellularity and inhibited differentiation of rat incisor odontoblasts. The pulp showed an increase

in cellularity. Pulp stones, with cellular and vascular inclusions were also found. The authors felt that these changes were similar to those observed in vitamin A and C deficiency.

Nakamoto and Wilson (1968) administered cortisone (40 mg/kg) to young rats and noted that tooth germ growth was retarded, the ameloblast and odontoblast layers were thinner, although the dentin was of normal thickness.

Davidovitch (1971) observed the mandibular condyles of cortisone treated (0.2 mg/gm of food or 1.9 mg/day) young rats to be sclerotic due to continuous matrix formation and lack of resorption. The cartilage cells were reduced in size and number in the proliferating and hypertrophic zones. The osteoblasts were also smaller and fewer in number. Cessation of cortisone therapy resulted in normal condyles.

### III. MATERIALS AND METHODS

Fifty-four female albino rats<sup>1</sup>, 90 days old, of the Sprague-Dawley strain were housed, two per cage. All animals were fed a normal pellet diet of Purina Lab Chow<sup>2</sup> for a period of 24 days. During this initial period all animals received tap water ad libitum. In order to minimize variability, only the investigators entered the animal room and with a minimum of handling of the animals. An automatic timing device was utilized to keep the room lighted from 7:00 A.M. to 7:00 P.M. and darkened from 7:00 P.M. to 7:00 A.M. The temperature, recorded automatically with a "tempscribe", ranged between 72° to 78°F.

Throughout the experiment the rats were visited at regularly scheduled intervals. On Monday the water was changed, on Wednesday the cage pans were changed, and the food supply was replenished. Body weights were taken on Friday beginning on the 25th day and ending on the day prior to sacrifice. All visits occurred between 5:00 to 7:00 P.M. with the exception of the sacrifice day.

Kodachrome slides were taken of the normal animals at the beginning of the experiment and periodically throughout the experiment as deficient signs began to appear: i. e., hyperemia of the ears and feet, periorbital conjunctivitis, dull hair, hair loss, size comparison of droppings and skin lesions. Also noted were the onset of hyperirritability and convulsions. Just prior to sacrifice while under anesthesia, slide photographs were taken of the teeth of

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<sup>1</sup>Purchased from ARS/Sprague-Dawley, Madison, Wisconsin.

<sup>2</sup>General Biochemicals, Chagrin Falls, Ohio.

representative animals in each of the six groups.

On the 25th day, the rats were divided into three groups: (A) 16 normals, (B) 18 controls, and (C) 20 deficient of magnesium (Mg). Group A continued to receive tap water and the normal Purina pellet diet containing 0.20% Mg and 1.20% calcium. Group B was given a pellet diet containing 0.081% Mg and 0.90% Ca and singly distilled water. Group C was started on a pellet diet containing 0.012% Mg and 0.89% calcium. These animals also received singly distilled water. On the 35th day, groups B and C continued to receive the single distilled water which, however, was now passed through a deionizing column. This procedure was instituted since signs of the deficiency were not evident and it was felt that this procedure would eliminate practically all traces of Mg from the water. On the 57th day, since no visible deficient signs were as yet present, the animals of group C were given a new Mg deficient diet containing 0.0051% Mg. By the 106th day all group C animals had manifested signs of the deficiency, therefore each of the three groups were divided. One-half of each group received daily subcutaneous injections of cortisone acetate<sup>3</sup>. The dosage employed was 3 mg/kg and the injections were alternated between the right and left flank. The other one-half of each group was similarly injected with normal saline.

The cortisone injections continued for a period of 15 days. The animals were sacrificed on the 121st day. Beginning at 7:00 A.M. and again at 1:00 P.M., one-half of each of the subgroups was injected subcutaneously with tritiated (3H) thymidine<sup>4</sup> (specific activity of 0.36 c/mM at a concentration

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<sup>3</sup>Donated by Merck and Co., Inc., West Point, Pa. 19486.

<sup>4</sup>Thymidine (methyl-3H) prepared by Schwartz-Mann Division of Becton-Dickinson and Co., Orangeburg, N.Y. and donated by Dr. Walter Kisieleski, Argonne National Laboratory, Argonne, Illinois.

of 1 mc/ml) and also with colchicine<sup>5</sup>. The dosage of 3H-thymidine was 0.6  $\mu$ c/gm and the dosage of colchicine was 1 mg/kg. The thymidine was injected into the left flank and the colchicine into the right. Thymidine is a base of DNA and is taken up by the nucleus during the S period of interphase and the isotope can be visualized through the use of radioautography or liquid scintillation. Colchicine stops mitosis at metaphase by interfering with spindle tubule formation, by retarding centromere division and by preventing centriole division. Chromatids do not separate from each other on time, so they continue coiling for a longer period than normal. Therefore metaphase chromosomes are shorter than normal. This inhibition of dividing cells in the metaphase stage aids the investigator in the counting of these cells. The colchicine powder was dissolved in chemically pure water immediately before injection.

Three hours later the rats were anesthetized with ether and then decapitated. The mandibles were removed from the head, the skulls were split midsagittally, excess tissue was trimmed away and each of these three tissue pieces was then placed in 10% formalin buffered with  $\text{CaCO}_3$ .

Following fixation, the teeth were then decalcified in a sodium citrate-formic acid solution, trimmed of the remaining excess tissue, washed in tap water for 24 hours and dehydrated. The maxillary incisors were then imbedded in paraffin, sectioned sagittally at 5 $\mu$  and stained with hematoxylin and eosin. Midsagittal sections through the cervical loop were used for the counting of mitotic figures.

Mitotic cell counts were made on four cell layers of the cervical (basal or labial) loop of both maxillary incisors. Specifically, these layers were the stratum intermedium, the preameloblast layer, the preodontoblast layer and the adjacent pulp cells. In addition, a thorough histological examination was made of the entire incisor and its supporting structures. The stratum intermedium and

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<sup>5</sup>Colchicine alkaloid U.S.P. purchased from Sigma Chemical Co.,

St. Louis, Missouri.

preameloblast layers were counted separately beginning at the base of the cervical loop and ending where cell division was no longer evident. Counting of the preodontoblast layer was started 0.09 mm from the base of the cervical loop and ended where the predentin layer began. Counting of pulp cells was started and ended as in the preodontoblast layer. An area extending 0.09 mm into the pulp from the preameloblast-preodontoblast junction was counted.

An American Optical binocular microscope with an objective lens (45x) and an ocular lens (10x) was used to make the counts. An ocular micrometer disc was used to count the preodontoblast and pulp cells. Precautions were taken to avoid counting overlapping areas twice. Adjacent sections of the incisors were not counted in order to avoid duplication. Four midsagittal sections were counted for each tooth.

The right mandibular incisor was selected for analysis utilizing the method of liquid scintillation counting. Under a binocular dissecting microscope (10x), a 2 mm length of the cervical or root end of each incisor was carefully dissected free. Special care was taken to keep the size of the tissue as constant as possible. The root ends were then taken from 75% ethyl alcohol, air dried for several hours and placed in a dessicator at room temperature for a period of four days after which they were individually weighed. The samples were then prepared for liquid scintillation counting by utilizing a Packard Tri-Carb sample oxidizer (Model 305). With this instrument the sample is automatically combusted. The tritiated water is then dispensed into a counting vial to which is added a measured amount of scintillator. Since with the use of this instrument, the steps in sample preparation are fully automatic, a very high degree of standardization is achieved which is not possible with conventional methods. The samples were then counted with a Packard Tri-Carb fully automatic liquid scintillation spectrometer (Model 3375). The counts were recorded as counts per minute. These were then converted to disintegrations per minute using the counting efficiency of the spectrometer, i. e. 37.60%, in the calculations. Both sample preparation and sample counting were carried out at

Argonne National Radiation Laboratory, Division of Biological and Medical Research, Argonne, Illinois.

The analysis of variance (F-test) and Student t-test were employed to statistically analyze the data. Differences between the groups of data were considered to be significant at the 5% level of probability.

#### IV. EXPERIMENTAL RESULTS

##### A. Gross Findings

On the 30th day of the experiment, or 5 days on the diet regimen, the amount and size of the stool was reduced in the controls and further reduced in the deficient rats as compared to the normals. By the 62nd day, or 5 days after the magnesium (Mg) deficient rats began receiving 0.0051% Mg in the diet, their stool was but one-third normal size and that of the control was only slightly larger than the deficient. This size relationship remained constant throughout the experiment.

By the 67th day, a greater hair loss was seen in all deficient rats as compared to the normals. Six control rats showed the same amount of hair loss as the deficient rats, while the remaining 12 showed only slightly more loss than the normals. By the 77th day, all control animals showed excessive hair loss, but by this time the deficient rats had even greater loss. This proportionate amount of loss remained fairly constant between the three groups throughout the rest of the experiment. The ventral surface showed a far greater loss than the dorsal.

On the 65th day, one deficient rat displayed a definite hyperemia of the ears, which extended from the base nearly to the tip (fig. 3). Two days later this hyperemia disappeared. On the 73rd day two deficient rats showed hyperemia of the ears and tail base and one had faint ear hyperemia, which, 4 days later was quite pronounced. Another deficient rat revealed slight ear hyperemia on the 79th day. By the 81st day, a normal rat developed a large hard mammary tumor from unknown causes and was removed from its cage and disposed of 6 days later. All deficient animals had experienced hyperemia of the ear by the 106th day. On the 84th day, one deficient rat, in addition to



the ear and tail base involvement, showed hyperemia of the hind paws and periorbital tissue (fig. 4). Two days later, another deficient had eye involvement also. The right eye of still another deficient had periorbital hyperemia on the 100th day. Once hyperemia of the eye appeared, it persisted throughout the experiment, in contrast to ear involvement which appeared, then disappeared only to recur later. Hyperemic signs were never observed in either the control or normal rats.

The fur of the normals remained glossy and thick throughout the entire experiment (fig. 2). The fur of the controls was nearly as glossy as the normals. By the 95th day, the deficient rats had dull fur and the hairs were brittle (fig. 5). An ulceration on the right shoulder of one deficient also appeared on this day.

Food and water consumption was reduced in the deficient rats by the 80th day and remained so throughout the experiment. Water consumption by controls was essentially the same as normal, but food consumption was definitely less, but not as low as in the deficient rats.

All normal and control animals displayed a contented and normal behavior throughout the experiment. By the 79th day, all deficient rats displayed nervousness in response to noise, handling or appearance of the investigators. One such deficient remained this way to the end of the study. This nervous activity would diminish to normal then reoccur. It consisted of hiding, running around the cage or slight jumping with occasional screeching. On the 113th day another deficient went into convulsions. It bit the investigator's glove, the body and tail of its cagemate, and the metal feeder. Immediately thereafter it lapsed into tetany. Subsequently, this rat exhibited only slight nervousness.

Two deficient rats developed body sores after the 106th day, one on the back and medial to the right ear, the other rat, near the tail on the back (fig. 5). None of these sores were severe. The right hind paw of a deficient rat was swollen and hyperemic on the 113th day, another showed the same

condition on the mid phalange of the right front paw on the 116th day and another deficient had similar involvement of the left fore paw on the 117th day.

The incisors were closely inspected prior to sacrifice. The normal labial surface was shiny and orange and the tip had an even edge (fig. 6). Some deficient incisors showed initial deficient signs as evidenced by pigmentation loss of the lower incisor which was a dull light orange (fig. 7). In more severe cases the incisal edge of the incisor was chipped, the orange upper incisor was dull instead of shiny and the lower incisor had almost total pigment loss and was white (fig. 8).

The weight gain of the normal and control rats was consistently higher than that of the deficient rats only after the diet of the latter was reduced to 0.005% of magnesium. Also in any given cage of normals, the heavier animals remained so throughout the experiment. With the control and deficient rats this was also the case, but some animals initially lighter were heavier by the end of the experiment.

Table VII shows the mean weight for each of the three groups. Each period represents a water or dietary change. Cortisone treatment was begun on the 109th day and was ended on the day preceding sacrifice. Initially from the 25th to the 60th day the mean weights of the three groups were essentially the same. By the 109th day, still no significant difference between the weight of the three groups was noted even though the deficient group showed only minimal weight gain since the 60th day. In the last period studied, after one-half the animals of each group had received cortisone for 11 days, the weights of the cortisone treated normal and control rats were almost identical as were these weights in the two saline groups. Both cortisone treated groups showed lower average weights than the non treated rats, but not significantly so. The weights of the cortisone treated and non treated deficient rats were essentially the same, and the mean weight of deficient rats was decidedly lower than that of either normal or control rats.

## B. Histological Findings

The tissues studied were the alveolar bone, periodontal ligament, enamel, dentin, enamel epithelium, enamel organ, odontoblasts and the pulp of the maxillary incisors (figs. 9, 10, 11, 13, 14, 17).

No consistent histologic differences were noted between the cortisone treated and non treated normal animals. The control animals showed mild pathology involving slight pulpal edema and very mild vacuolization of the enamel epithelium, but again no distinction was seen between the cortisone treated and nontreated animals.

The changes seen in the incisors of deficient animals ranged from moderate to severe. The non-treated deficient group revealed stratified pulp stones in some instances and the size of the pulp cavity was reduced (figs. 15, 19). Evidence of pulpal edema was manifested by a reduction in pulp cell density. Some capillaries were undergoing calcification (fig. 19). In severe cases the pulp cells became abnormal in shape, lacked their processes and stained fainter than normal pulp cells. In some instances pulpal hyperemia was seen as evidenced by excessive numbers of blood vessels which were swollen and engorged with erythrocytes. Both labial and lingual dentin was stratified and thicker than normal (fig. 15). The stratification was made up of alternate axially oriented hematoxylin and eosin stained bands. This phenomenon has been described by Klein et al. (1953), Irving (1941) and Becks and Furuta (1942). As seen in the normal rats, the lingual dentin was thicker than the labial (fig. 15). However, instead of a discrete, even, pulpal border, the dentin was irregular and indistinct with spurs extending into the pulp (fig. 21). The dentin showed invaginations containing pulpal and odontoblastic tissue (fig. 21). Also noted were islands of pulpal tissue isolated within the dentin (fig. 22). The dentin stratification was wavy and irregular and stained unevenly. It was the overgrowth of dentin that reduced the size of the pulp cavity. Just distal to the cervical loop in the area of initial dentin and pre-dentin formation, there was a sudden thickening of both of these tissues

instead of the gradually widening as seen in the normal (fig. 12). In one animal, three distinct, separate, small, round voids were seen in the incisal area of the dentin. In some instances epithelial pearls were seen in the dentin bordering the enamel epithelium (figs. 27, 28). One incisor showed an extreme breakdown of the dentin adjacent to the degenerated enamel epithelium (figs. 27, 28). The enamel epithelium appeared to be active in this breakdown process. We are not aware of this observation having been reported in the literature. The non-treated deficient animals did not show folded cervical dentin as reported by Becks and Furuta (1942). The odontoblastic layer was degenerated in some incisors and completely absent in others (figs. 12, 25, 26). In some teeth, the alveolar bone was stratified more than normal and severe resorptive changes were evidenced in one incisor by the appearance of smooth, round knobby bone adjacent to the lingual dentin (figs. 15, 16). The periodontium was widened and edematous (figs. 15, 16).

The enamel matrix of one tooth appeared to be invaded by the enamel epithelium (figs. 29, 30). Many enamel pearls were present within the epithelium. This finding is similar to that of Becks and Furuta (1941), but this condition in our rats appeared more severe with the adjacent enamel matrix completely broken up into pearls. There were instances of isolated enamel defects with a V-shaped void in the outer surface, indicating enamel hypoplasia. In some incisors the ameloblasts were completely degenerated, and it appeared that enamel formation was absent.

The enamel epithelium, comprised of an inner ameloblast layer with an outer papillary layer, showed changes ranging from mild to a complete destruction and absence (figs. 18, 20). Mild changes were indicated by the presence of vacuoles in both layers. Other incisors revealed shortened ameloblasts and a taller papillary layer with thin villus-like projections. More severe changes included a less distinct and shortened papillary layer and ameloblasts that went from typical columnar to a flattened, irregular appearance (figs. 18, 20). These cells then atrophied to a thin single cuboidal layer, and finally to

squamous shape. The papillary layer was still recognized in these teeth (figs. 10, 12). The most severe change was a complete absence of the enamel epithelium in certain areas of the tooth (fig. 10). At the cervical level of some incisors, the normal morphology was replaced by a thin dark line which may represent a condensation of degenerate cells (fig. 25). The connective tissue was adjacent to the degenerate epithelium, a finding reported by Becks and Furuta (1939).

The basal (labial) loop showed changes ranging from mild cellular atrophy to severe hypoplasia, disorganization and degeneration (figs. 12, 23, 24, 26). The stratum reticulum and stratum intermedium appeared to be more sensitive to the deficiency than the preameloblast, preodontoblast or pulpal layers. This was evidenced, in some instances, by complete replacement of these two layers with fibrous tissue, while the other cell layers were still present (figs. 23, 24). In the severest cases, even the preameloblast and preodontoblast layers were unrecognizable and only bizarre pulp cells were evident (fig. 12). These pulp cells were not round in appearance as reported by Becks and Furuta (1942), indicating, in their study, that degeneration may have been more extreme. In mild cases, the overall basal loop was somewhat smaller but fairly normal in appearance. Other incisors showed more severe changes so that the loop appeared shorter, narrower and stained fainter. Instead of a rounded club-shaped loop, the tip became pointed. Finally, in the severest instances, the loop was shorter and thinner than normal and stained an intense blue with no recognizable cells (fig. 12).

The cortisone treated deficient incisor showed the same pathological changes as the non-treated deficient incisor. One treated animal revealed incisors with severely folded dentin beginning at the level of the cervical loop area (fig. 26). This finding was reported by Becks in 1941. This was considered to be the severest change noted in the present study. Surprisingly, even these incisors showed no islands of pulpal calcification or stones as was frequently observed in the non-treated deficient incisor.

### C. Mitotic Activity of the Basal Loop

Tables I through VI summarize the results. In all three diet groups, it can be noted that the average number of mitoses is the least in the preodontoblast layer, to be followed by the stratum intermedium, the preameloblast layer and finally the pulp (fig. 10). This result is directly related to the total number of cells counted for each of the cell layers.

As shown in Tables II through VI, statistical treatment of the data by means of the three way analysis of variance shows, in all four cell layers studied, that both the diet and cortisone exerted a highly significant effect ( $P < .001$ ) on the mean number of mitoses in the three groups of animals studied. The analysis also revealed a significant difference between these means at 7 A.M. vs those at 1 P.M. in all cell layers studied except the pulp. This circadian or diurnal effect is not observed when the data of the combined cell layers are analyzed, indicating the overriding influence of the pulp cells. When the effects of diet and cortisone on incisor cell proliferation are considered together, only the preameloblast layer reveals a significant difference ( $P < .01$ ). This finding indicates that the effect of these two influences together is not as great as each one separately. The influence of diet and time (circadian effect) interaction on cell proliferation shows a significant effect on the stratum intermedium, the preameloblasts and the combined layers but not on the preodontoblasts or pulp. When the influence of cortisone is interacted with the circadian effect, only the pulp cells show a significant difference ( $P < .001$ ). A statistical comparison of the interactive influences of the three variables (diet, cortisone and time) on cervical loop mitosis shows a significance in only the preodontoblast and pulp cell layers.

In an evaluation of the individual differences in the mean number of mitoses between the dietary, cortisone and time groups, Table I shows that the numerical values of the normal animals are generally greater than those of the control rats. These differences, however, are of small magnitude. On the other hand, the differences between the averages of deficient animals

compared to normal or control values are quite large.

A statistical comparison (t-test) between the averages of cortisone treated and non-treated animals within each of the three dietary groups, shows, in some instances, significantly more mitotic figures in the cortisone treated animals (e.g. normal, 7 A.M., stratum intermedium  $P < .05$ ). However, most comparisons reveal no statistically significant difference ( $P > 0.10$ ). Nevertheless, in nearly every comparison, it can be noted that the values of the cortisone treated animals are numerically greater than their saline treated counterparts. Therefore, one might conclude that the strong tendency is for the cortisone treated animals to show a higher number of mitoses in the cells of the cervical loop. This tendency reached the level of high statistical significance in all cell layers studied when the overall activity was evaluated with the three way analysis of variance. Numerically, the deficient animals showed essentially no difference between the cortisone treated and non-treated values. When the cell layers were combined, the averages of the cortisone treated animals were seen to be more, in every instance, than the comparative non-treated values. Again these comparisons were not always statistically significant. A strong circadian effect is not readily apparent by inspection of the data in Table I. However, the results of the three way analysis of variance reveals the presence of this effect in every layer but the pulp on an overall statistical basis as shown in Tables II through VI.

Table VIII reports the results of the uptake of  $^3\text{H}$ -thymidine by the root tip of the right mandibular incisor as measured by the liquid scintillation spectrometer. In every instance, the mean activity (disintegrations per sample or disintegrations per milligram) of the cortisone treated root tips is greater than that of the non-treated groups. In no comparison except one (1 P.M., control animals) were the differences statistically significant. However, the strong tendency, based on the average values, is toward an overall significant increase in the uptake of  $^3\text{H}$ -thymidine under the influence of cortisone. Moreover, the results of this data very significantly reinforce the data on mitotic

cell counts in comparing the cortisone treated and non-treated groups. In addition, as also shown with the mitotic counts, the deficient incisors showed much less activity as compared to the normal and control teeth. A significant observation, not readily apparent with the mitotic count data, shows more disintegrations per minute in the control incisors than in the normal. On the basis of the average activity, the presence of a circadian effect in the uptake of  $^3\text{H}$ -thymidine is not apparent.



## V. DISCUSSION

In discussing the results of this study, one must keep in mind that the three factors influencing the incisor namely; diet, cortisone and time, are dependent on the maturation level of the animal. That is, a very young, growing rat (or any other organism) is more sensitive to a magnesium deficiency than an older, more mature animal (Aikawa, 1963; Kiely, 1967). This is because the young animal is undergoing rapid growth and tissue maturation while in the older animal these processes are minimal and the adult tissue is concerned with only maintenance and replacement. When Kiely (1967) studied magnesium deficiency in 30 day old rats, the majority died before sacrifice and the data on mitotic activity was incomplete. Therefore, in order to minimize the rate of mortality, 90 day old rats were chosen for this study. These animals proved to be extremely hearty and responded somewhat slowly to magnesium deprivation. Nevertheless, definite, gross changes were eventually established in all deficient animals and classic histologic changes were also observed in the incisor.

Gagnon, Schour and Patras (1942) noted a slight weight reduction in young rats fed a magnesium deficient diet. Martindale and Heaton (1964) noted a weight loss in adult magnesium deficient rats. Kiely (1967) observed, in young rats, a very significant weight loss with both the magnesium deficient and supplemented control diets and a larger difference was noted between the final weights. Each of the three groups had a subgroup treated with cortisone which lost more weight than the non-treated groups. However, the deficient rats showed little loss of weight due to cortisone. Our study showed the weight gain of the controls to be the same as the normal group.

However, Kiely's control rats were supplemented with 0.063% magnesium while our rats were given 0.081%. Also, our rats were much older than those used in Kiely's study, again pointing up the important relationship between age of the animal and the severity of the response to the magnesium deficiency. The weights of our deficient rats were in keeping with those of controls and normals until the 0.0051% diet was started whereupon the weight gain decelerated below that of the normal and controls. At the end of the study, the average weight of deficient rats was still not significantly less than the two other groups although there was a numerical difference. As in Kiely's study, our cortisone treated animals weighed less than the saline treated ones and the deficient rats showed the least amount of weight loss after cortisone treatment. Parmer, Katonah and Angrist (1951) and Nakamoto and Wilson (1968) demonstrated growth inhibition and weight loss in rats given cortisone. This effect of cortisone has been reported innumerable times in the literature. Apparently, in our animals, the effects of the magnesium deficiency served to prevent additional weight loss during cortisone treatment. However, if our injections had continued, a more severe weight loss would most likely have occurred. Kiely (1967), after treating adult magnesium deficient rats with cortisone for a period of nine weeks, noted little initial weight loss. However, by the end of treatment, a significant loss of weight had occurred. In addition, he found that cortisone given over a four week period to young severely depleted magnesium deficient rats, resulted in little weight loss when compared to untreated deficient rats.

Statistical evaluation with the analysis of variance, showed diet to be a highly significant factor in influencing the proliferative activity of the cervical loop. Cortisone treatment was also found to be a significant factor in the overall increase in cell division. Time was shown to have a significant influence on the rate of cell proliferation for all layers studied except the pulp. Interactions of these variables showed some significance but generally the effect of the individual variable had more effect than their

combined action.

The cell counts of the deficient incisors for a given layer were essentially equal between the cortisone treated and non-treated rats. This would indicate that the diet deficiency played the dominate role and that the cortisone effect was secondary. It appears that the cell layers of magnesium deficient rats respond somewhat poorly to cortisone; the normal and control incisors seemed to respond more positively to the effects of cortisone than the deficient teeth. However, uptake of  $^3\text{H}$ -thymidine in the deficient incisors was much greater in the cortisone treated teeth, thus strongly indicating a stimulation in the rate of DNA synthesis in these deficient incisors. One might expect that these two methods for determining the proliferative activity of a tissue would yield similar results. The possibility exists that with the reduced numbers of mitoses brought about by the deficiency, more extensive cell counts were needed in order to detect differences between cortisone treated and non-treated deficient incisors. With the scintillation counting procedure, total proliferative activity of the growing root end was analyzed. Also, in the total pool of dividing cells, many more cells are undergoing synthesis of DNA than are engaged in mitosis. Therefore, the amount of activity as measured by the scintillator might be expected to yield, in the case of the deficient incisors, more complete information than the activity as indicated by the mitotic counts. At any rate, the cell counts showed no inhibition of mitoses by cortisone; an observation reported for other tissues, eg. epidermis and liver. It has been shown by Roberts et al. (1952) that cortisone does not inhibit mitoses in all tissues. Kiely (1967) found that the eruption rate of the rat incisor increased with cortisone administration and so did the rate of mitosis in the normal incisor (Domm and Kiely, 1968) and DNA synthesis in the incisor of adrenalectomized rats treated with cortisone (Kiely and Domm, 1973). These findings on eruption rates were substantiated earlier by Parmer, et al. (1951), Domm and Marzano (1954) and others. These studies do not, however, conclusively establish the mechanism of action of

cortisone on eruption. The possibility exists that the investing tissues, namely the alveolar bone, periodontal ligament and gingiva offer less resistance to the forces of tooth eruption under the influence of cortisone. Glickman, et al. (1953) noted decreased alveolar bone activity, and an edematous periodontal ligament in mice given cortisone. Applebaum and Seelig (1955) observed alveolar bone loss and pulpal blood vessel dilatation in young rats given cortisone. Goldsmith and Ross (1956) described increased alveolar bone formation, ameloblast degeneration and periodontal ligament disorganization in cortisone treated rats. They also observed premature incisor differentiation of the incisor. Blackey and Johannessen (1964) found that cortisone inhibited jaw development, dentin apposition and overall body growth. Odontoblast differentiation was inhibited but increased cellularity was evident in the rat incisor as seen by Anneroth and Bloom (1966). The pulp had increased cellularity. Nakamoto and Wilson (1968) found cortisone treated young rats to have retarded tooth germ growth and thinner ameloblast and odontoblast layers. Fleming (1953) found that cortisone treated tooth germs transplanted into the anterior eye chamber of guinea pig adults vascularized more rapidly than controls, grew faster initially but after four days fell behind controls, and did not attach to the iris of the host as in the controls. The results of these studies on the effects of cortisone on oral tissues are somewhat conflicting. This may be due to a number of variable factors: for example, dosage of cortisone employed, duration of treatment, age and species of the experimental animal. However, the investigators seem to agree that cortisone has an edematous, disorganizing effect on the periodontium. When one reviews the effects of cortisone on bone and body growth in the rat, there is total agreement that the rate of body growth is decreased (Parmer, et al. 1951; Fleming, 1953; D'Arcy and Howard, 1961; Nakamoto and Wilson, 1968; Davidovitch, 1971), also bone growth is disrupted and retarded (Sissons and Hadfield, 1955; Hulth and Olerud, 1963; Davidovitch, 1971). The net effect of bone growth inhibition and periodontal tissue disruption might

conceivably be less resistance to the forces of tooth eruption. However, in our animals one could distinguish no histological differences between cortisone treated and non-treated incisors. Moreover, cortisone treated animals did show an increase in proliferative activity. In the deficient group, basically the same histopathology was noted for the cortisone treated and non-treated rats. Therefore, one might conclude, on the basis of our results, that the stimulatory effect of cortisone on incisor eruption may be, at least in part, the result of its stimulatory effect on mitosis. Cortisone has been shown by Kiely (1967) to accelerate this rate even in magnesium deficient rats though not to the level of control values. In our study, cortisone enhanced the uptake of  $^3\text{H}$ -thymidine in deficient incisors though, again, not to normal or control values. Whether this positive effect of cortisone on the cervical loop tissue of the incisor is a direct one or is indirect (i. e., mediated through the investing tissues e.g. periodontal ligament, alveolar bone) remains to be determined.

Time of sacrifice was a significant influence on mitosis in three of the four layers studied. However this effect was only more significant than the effect of cortisone in the stratum intermedium while in the other layers it was not as significant a factor as either diet or cortisone. Nevertheless, circadian mitotic rhythms are known to be present in a variety of tissues and this effect should be considered in studies on cell division in the incisor.

Clinically, the behavior and appearance of the normal and control rats was quite similar. However, the stool was smaller in the control rats and there was more hair loss. These changes can undoubtedly be attributed to a decrease in dietary magnesium.

The cortisone treated deficient group showed a definite lessening of skin and eye hyperemia so that by the 13th day of cortisone treatment, none of these animals had hyperemia or skin lesions, an observation previously reported by Kiely (1967). Three non-treated deficient rats still had signs of ear hyperemia and two of these had body sores. The fur of the cortisone treated

and non-treated deficient rats appeared the same; it was not glossy and thick as in the normals.

It was also noted that the untreated deficient animals displayed individual responses to magnesium deficiency, that is, some were more resistant to the deficiency than others. Not all developed paw or eye hyperemia, and only one experienced convulsions. Some were more nervous than others. The gross changes following magnesium deficiency in our study have been classically reported by Kruse, Orent and Mc Collum (1932) and Watchorn and Mc Cance (1937).

Since our rats were more mature than those of Becks and Furuta (1939, 1941 and 1942), we noticed less histopathology in the incisors than did these authors. Had our study continued for a longer period, undoubtedly more striking changes would have been evident in the incisors of the deficient. However, we would have risked an increased mortality rate.

In the deficient group, instances of incisor depigmentation and whitishness were noted. The lower incisors always showed these changes earlier and more severely than did the uppers. This correlates well with the study of Domm and Marzano (1954) who described a faster eruption rate of the lower as compared to the upper incisor. Thus, the lower incisor may be a more actively growing organ than the upper incisor and therefore may be more sensitive to deprivation of magnesium. Anatomically, the lower is longer than the upper. With these facts in mind, it is suggested that the lower incisor may be more useful in many studies dealing with dietary or drug effects in rats.

The results of our study clearly indicate that proper levels of magnesium must be maintained for normal growth, development and maintenance of the incisor and its supporting and investing tissues. The abnormal changes, ranging from mild atrophy to hypoplasia and degeneration, seen in the enamel epithelium, enamel organ, odontoblasts, pulp, periodontium and alveolar bone together with the resultant disturbances in dentin and enamel calcification

have been described earlier by Klein, Orent and Mc Collum (1935), Watchorn and Mc Cance (1937), Becks and Furuta (1939, 1941, 1942), Irving (1940), Gagnon, et al. (1942), Bernick and Hungerford (1965), Trowbridge and Seltzer (1967), Kiely (1967), Trowbridge and Jenks (1968), and Trowbridge, et al. (1971).

Magnesium is a necessary part of structural RNA which is required for protein synthesis (Wacker, 1969). It is also vital for the maintenance of ribosomal structure. Magnesium is needed for each reaction leading to protein synthesis; not only for structural integrity but also for activation of specific enzymes. Among the latter are alkaline phosphatase, ATPase, muscle enolase, creatinine kinase and pyruvate kinase. These enzyme systems are important for growth, maturation and calcification of dental and investing tissues (Bernick and Hungerford, 1965; Trowbridge et al. 1967, 1968, 1971). Pathology seen as a result of magnesium deficiency is most certainly based upon the malfunction or inaction of vital enzymes and interference in the structural integrity of basic molecules.

Therefore, the results of our investigation have served to lend confirmation to the aforementioned studies, which show severe disturbances in the tissues of and surrounding the rat incisor. In addition, however, we have directly shown that magnesium deficiency significantly inhibits the rate of cell-proliferation in the formative tissues of the incisor. Also, our study has revealed a stimulation in the proliferative activity of these tissues in the normal, magnesium supplemented and to a lesser extent, in the magnesium deprived rats following cortisone treatment. Kiely (1967), Duckworth and Godden (1940) and Gagnon et al. (1942) have reported a marked inhibition in the rate of incisor eruption as a result of magnesium deficiency and Kiely (1967) has shown that cortisone accelerates this rate; however, to a level still below control values. Others (Parmer, Katonah and Angrist, 1951; Domm and Marzano, 1954; Garren and Greep, 1960) have observed cortisone to restore incisor eruption to normal in the case of gland removal and to

increase this rate in normal rats. Our results, then, appear to lend strong support to the postulation that cortisone exerts a stimulatory effect on the growing incisor with a resultant increase in eruption. The observation that cortisone does not restore eruption to normal in magnesium deficient animals is consistent with our finding that cell proliferation was increased with cortisone injection but to levels below control values.

It could be theorized, then, that cortisone may tend to normalize the tissues of the incisor by activating magnesium pools in bone and soft tissue therefore allowing distribution to various sites including the incisor. The ability of cortisone to "normalize" the incisor tissues would then be dependent on the amount of available magnesium in the body reserves. In the case of very young or chronically deficient animals, this process would, of necessity, be less effective than in the case of adult, short-term deficient or magnesium supplemented animals.



## VI. SUMMARY AND CONCLUSIONS

Fifty-four female, albino rats, 90 days old, of the Sprague-Dawley strain were randomly selected in order to study the effect of cortisone on the magnesium (Mg) deficient incisor. During an acclimation period of 25 days, all animals received tap water and a normal diet consisting of Purina lab chow. The animals were then divided into a normal group, a control (Mg supplemented) and a diet deficient group. After the appearance of classic signs of the deficiency (eg. hyperemia of the ears, irritability, etc.) each group was subdivided so that one half received daily injections of cortisone. After 15 days, the animals were given a single injection of colchicine and  $^3\text{H}$ -thymidine at 7 A.M. or 1 P.M. and sacrificed three hours later. Mitotic cell counts were made on the stratum intermedium, preameloblasts, preodontoblasts and pulp cells of the labial loop. The lower, right incisor was utilized to study the uptake of  $^3\text{H}$ -thymidine by means of the liquid scintillation spectrometer. A detailed histological examination of the upper incisor was also made. The results of the study are summarized as follows:

1. The control (supplemented with 0.081% Mg) animals showed normal growth and development. Their weight, appearance and general activity was normal. Histologically, the maxillary incisor appeared normal except for slight pulpal edema, minimal vacuolization of the enamel epithelium and some random capillary degeneration. Some hair loss and a decrease in stool size was also noted.
2. A diet of 0.0051% Mg was needed to bring about signs of the deficiency, as animals maintained on a deficient diet containing 0.012% Mg for 35 days revealed the same appearance as control

rats. Clinically, these animals had hyperemia of the ears, paws and periorbital tissue. Epidermal sores, hair loss, small stool, hyperirritability, convulsions (one case), decreased weight gain, depigmented and whitened mandibular incisors were also noted. The mandibular incisors showed more extensive and earlier defects than the maxillary incisors.

Histologically, the upper incisors of the Mg deficient animals revealed mild to severe disturbances, which indicated an individual response of animals to the diet. Some incisors showed intact cervical loops that were shorter and narrower than normal with mild cell atrophy; in others, the cervical loop was degenerated and had connective tissue invasion. The enamel epithelium showed vacuolization. Non-treated deficient animals revealed pulp stones, stratified dentin, pulpal hyperemia, swollen periodontium, capillary degeneration and calcification, pulpal edema, dentin spurs into the pulp, knobby alveolar bone with increased stratification, and complete loss of enamel epithelium architecture. Widening of predentin and dentin, epithelial invasion and destruction of dentin and enamel were also seen. The cortisone treated incisors showed no pulpal stones, but one case of dentin folding was seen. All other changes noted for the non-treated animals were also seen in the cortisone treated rats. The stellate reticulum and stratum intermedium appeared more sensitive to magnesium deprivation than the preameloblast, preodontoblast and pulpal layers. This was evidenced, in extreme cases, by connective tissue invasion and destruction of these two layers.

3. The deficient groups showed, by far, the slowest mean weight gain. The weights of normals and controls were essentially equal.

4. The three way analysis of variance revealed that the effects of diet, cortisone administration and time of sacrifice significantly influenced the rate of mitosis in all cell layers studied. Diet appeared to have the greatest effect. Cortisone exerted less effect, but this effect was, nevertheless highly significant. Statistically, the interaction of these three variables generally showed less effect on mitosis than did each one separately.
5. In all cell layers studied, the average number of mitoses in the normal and control animals was much higher than in deficient. Normal values tended to be higher than those of controls. Cortisone treated values, though not always statistically significant, were consistently greater in both normal and control incisors than the non-treated averages. In the deficient animals, no difference was noted in the average number of mitoses between cortisone treated and non-treated incisors.
6. In every instance, the average rate of uptake of  $^3\text{H}$ -thymidine by the root tip of the mandibular incisor was greater in the cortisone treated animals as compared to the non-treated group. The highest amount of radioactivity was found in the root tips of controls, and the least was seen in the deficient animals.
7. It is concluded that level of animal maturation, duration of Mg deprivation, individual animal differences and types of tissue studied are factors that are operable and accountable for the variability observed in responses due to Mg deprivation.
8. Again, it has been conclusively shown that adequate amounts of Mg are necessary for the growth, development and maintenance of the maxillary incisor and its investing tissues.

9. The results are reviewed in comparison to current theories on the effect of Mg deficiency on the mitotic activity and histology of the maxillary incisor treated with cortisone. It is concluded that our study supports the findings of previous studies which show that the tissues of the maxillary incisor are seriously affected by deficient levels of Mg and that cortisone has a positive influence on the proliferative activity of the incisor in normal, Mg supplemented and Mg deficient rats.

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TABLE I

Mean Number of Mitoses in the Cervical Loop of the Maxillary Incisor  
of Normal, Control<sup>1</sup> and Magnesium Deficient Female Rats

| Group     | Time <sup>2</sup> | No. Rats | No. Obs. | Strat.            | Inter.            | Preamel. |       | Preodont. |                   | Pulp  |                   | Total  |                    |
|-----------|-------------------|----------|----------|-------------------|-------------------|----------|-------|-----------|-------------------|-------|-------------------|--------|--------------------|
|           |                   |          |          | Mean <sup>3</sup> | S.D. <sup>4</sup> | Mean     | S.D.  | Mean      | S.D.              | Mean  | S.D.              | Mean   | S.D.               |
| Normal    |                   |          |          |                   |                   |          |       |           |                   |       |                   |        |                    |
| Cortisone | 7 AM              | 4        | 26       | 16.42             | 5.33*             | 30.92    | 6.10* | 11.38     | 5.65              | 43.92 | 17.15             | 102.65 | 25.06              |
| Saline    |                   | 4        | 28       | 13.21             | 5.60              | 23.00    | 7.81  | 11.04     | 5.04              | 44.07 | 13.45             | 91.32  | 20.49              |
| Cortisone | 1 PM              | 4        | 20       | 20.00             | 5.37              | 36.55    | 5.92  | 13.35     | 4.74              | 54.90 | 15.29             | 124.80 | 18.91              |
| Saline    |                   | 3        | 20       | 19.20             | 5.28              | 34.10    | 8.28  | 8.05      | 3.76 <sup>o</sup> | 34.75 | 8.96 <sup>o</sup> | 96.10  | 17.18 <sup>o</sup> |
| Control   |                   |          |          |                   |                   |          |       |           |                   |       |                   |        |                    |
| Cortisone | 7 AM              | 5        | 32       | 15.09             | 4.88              | 26.31    | 6.95  | 11.56     | 4.06 <sup>o</sup> | 41.94 | 15.27*            | 94.91  | 24.54              |
| Saline    |                   | 4        | 28       | 13.36             | 4.22              | 24.00    | 6.29  | 8.07      | 2.88 <sup>o</sup> | 34.50 | 10.56             | 79.93  | 13.46              |
| Cortisone | 1 PM              | 5        | 36       | 16.69             | 5.09              | 26.58    | 6.18  | 8.94      | 2.92              | 40.75 | 15.00             | 92.97  | 22.15              |
| Saline    |                   | 4        | 16       | 17.31             | 7.52              | 23.69    | 9.45  | 7.88      | 4.70              | 39.50 | 12.34             | 88.33  | 29.68              |
| Deficient |                   |          |          |                   |                   |          |       |           |                   |       |                   |        |                    |
| Cortisone | 7 AM              | 5        | 40       | 8.43              | 2.42              | 20.50    | 5.36  | 6.15      | 2.90              | 16.83 | 7.14              | 51.90  | 11.25              |
| Saline    |                   | 5        | 36       | 8.42              | 4.14              | 19.72    | 3.95  | 5.50      | 3.09              | 15.64 | 5.13              | 49.28  | 10.46              |
| Cortisone | 1 PM              | 5        | 36       | 8.67              | 3.51              | 19.25    | 6.91  | 5.69      | 2.98              | 18.64 | 9.04 <sup>+</sup> | 52.25  | 18.31              |
| Saline    |                   | 5        | 36       | 9.14              | 4.86              | 19.56    | 7.24  | 5.75      | 2.39              | 13.53 | 4.88 <sup>+</sup> | 47.97  | 15.44              |

<sup>1</sup>Mg supplemented

<sup>2</sup>Colchicine administration

<sup>3</sup>Per tissue section

<sup>4</sup>Standard Deviation

\*Differences significant at 0.05 level or less

<sup>+</sup>Differences significant at 0.01 level or less

<sup>o</sup>Differences significant at 0.001 level or less

All other differences between means not significant at 0.10 level or greater.

TABLE II  
Stratum Intermedium  
Three Way Analysis of Variance

| Source of Variation | Sum of Squares | DF <sup>4</sup> | Mean Square | F Value | P Value <sup>5</sup> |
|---------------------|----------------|-----------------|-------------|---------|----------------------|
| A <sup>1</sup>      | 4868.25        | 2               | 2434.13     | 108.24  | < .001               |
| B <sup>2</sup>      | 98.72          | 1               | 98.72       | 4.39    | < .05                |
| C <sup>3</sup>      | 343.08         | 1               | 343.08      | 15.26   | < .001               |
| AB                  | 59.23          | 2               | 29.62       | 1.32    | > .10                |
| AC                  | 392.93         | 2               | 196.46      | 8.74    | < .001               |
| BC                  | -0.61          | 1               | -0.61       | -0.03   | > .10                |
| ABC                 | 41.23          | 2               | 20.62       | 0.92    | > .10                |
| <u>Within Cell</u>  | <u>7691.14</u> | <u>342</u>      | 22.49       |         |                      |
| TOTAL               | 13,493.90      | 353             |             |         |                      |

<sup>1</sup>Diet<sup>4</sup>Degrees of Freedom<sup>2</sup>Cortisone<sup>5</sup>Probability<sup>3</sup>Time

TABLE III  
Preameloblasts  
Three Way Analysis of Variance

| Source of Variation | Sum of Squares | DF <sup>4</sup> | Mean Square | F Value | P Value <sup>5</sup> |
|---------------------|----------------|-----------------|-------------|---------|----------------------|
| A <sup>1</sup>      | 6710.56        | 2               | 3355.28     | 76.65   | < 0.001              |
| B <sup>2</sup>      | 504.75         | 1               | 504.75      | 11.53   | < 0.001              |
| C <sup>3</sup>      | 236.25         | 1               | 236.25      | 5.40    | < 0.05               |
| AB                  | 449.44         | 2               | 224.72      | 5.13    | < 0.01               |
| AC                  | 1453.31        | 2               | 726.66      | 16.60   | < 0.001              |
| BC                  | 39.00          | 1               | 39.00       | 0.89    | > 0.10               |
| ABC                 | 99.06          | 2               | 49.53       | 1.13    | > 0.10               |
| Within Cell         | 14,970.69      | 342             | 43.77       |         |                      |
| TOTAL               | 24,463.06      | 353             |             |         |                      |

<sup>1</sup>Diet<sup>4</sup>Degrees of Freedom<sup>2</sup>Cortisone<sup>5</sup>Probability<sup>3</sup>Time

TABLE IV  
Preodontoblasts  
Three Way Analysis of Variance

| Source of Variation | Sum of Squares | DF <sup>4</sup> | Mean Square | F Value | P Value <sup>5</sup> |
|---------------------|----------------|-----------------|-------------|---------|----------------------|
| A <sup>1</sup>      | 1733.68        | 2               | 866.84      | 62.80   | < 0.001              |
| B <sup>2</sup>      | 197.32         | 1               | 197.32      | 14.30   | < 0.001              |
| C <sup>3</sup>      | 56.93          | 1               | 56.93       | 4.12    | < 0.05               |
| AB                  | 73.46          | 2               | 36.73       | 2.66    | > 0.10               |
| AC                  | -2.19          | 2               | -1.09       | -0.08   | > 0.10               |
| BC                  | 14.16          | 1               | 14.16       | 1.03    | > 0.10               |
| ABC                 | 200.38         | 2               | 100.19      | 7.26    | < 0.001              |
| Within Cell         | 4720.49        | 342             | 13.80       |         |                      |
| TOTAL               | 6994.19        | 353             |             |         |                      |

<sup>1</sup>Diet<sup>4</sup>Degrees of Freedom<sup>2</sup>Cortisone<sup>5</sup>Probability<sup>3</sup>Time

TABLE V  
Pulp Cells  
Three Way Analysis of Variance

| Source of Variation | Sum of Squares    | DF <sup>4</sup> | Mean Square | F Value | P Value <sup>5</sup> |
|---------------------|-------------------|-----------------|-------------|---------|----------------------|
| A <sup>1</sup>      | 57085.69          | 2               | 28542.84    | 210.55  | < 0.001              |
| B <sup>2</sup>      | 2898.88           | 1               | 2898.88     | 21.38   | < 0.001              |
| C <sup>3</sup>      | 11.69             | 1               | 11.69       | 0.09    | > 0.10               |
| AB                  | -177.69           | 2               | -88.84      | -0.66   | > 0.10               |
| AC                  | 105.56            | 2               | 52.78       | 0.39    | > 0.10               |
| BC                  | 1349.44           | 1               | 1349.44     | 9.95    | < 0.01               |
| ABC                 | 1330.25           | 2               | 665.13      | 4.90    | < 0.01               |
| Within Cell         | 46362.38          | 342             | 135.56      |         |                      |
| <u>TOTAL</u>        | <u>108,966.00</u> | <u>353</u>      |             |         |                      |

<sup>1</sup>Diet<sup>4</sup>Degrees of Freedom<sup>2</sup>Cortisone<sup>5</sup>Probability<sup>3</sup>Time

TABLE VI  
Total Cells  
Three Way Analysis of Variance

| Source of Variation | Sum of Squares | DF <sup>4</sup> | Mean Square | F Value | P Value <sup>5</sup> |
|---------------------|----------------|-----------------|-------------|---------|----------------------|
| A <sup>1</sup>      | 184388.00      | 2               | 92194.00    | 256.97  | < 0.001              |
| B <sup>2</sup>      | 9996.00        | 1               | 9996.00     | 27.86   | < 0.001              |
| C <sup>3</sup>      | 534.00         | 1               | 534.00      | 1.49    | > 0.10               |
| AB                  | 2042.00        | 2               | 1021.00     | 2.85    | > 0.10               |
| AC                  | 4139.00        | 2               | 2069.50     | 5.77    | < 0.01               |
| BC                  | 518.00         | 1               | 518.00      | 1.44    | > 0.10               |
| ABC                 | 1443.00        | 2               | 721.50      | 2.01    | > 0.10               |
| Within Cell         | 122,699.00     | 342             | 358.77      |         |                      |
| TOTAL               | 325,759.00     | 353             |             |         |                      |

<sup>1</sup>Diet<sup>4</sup>Degree of Freedom<sup>2</sup>Cortisone<sup>5</sup>Probability<sup>3</sup>Time

TABLE VII

Mean Weights<sup>1</sup> of Normal, Control and Magnesium Deficient Female Rats

| Day | N <sup>2</sup> | Normal    |                    | N  | Control <sup>4</sup> |       | N  | Deficient |       |
|-----|----------------|-----------|--------------------|----|----------------------|-------|----|-----------|-------|
|     |                | Mean      | S. D. <sup>3</sup> |    | Mean                 | S. D. |    | Mean      | S. D. |
| 25  | 16             | 234.75    | 16.75              | 18 | 232.44               | 12.23 | 20 | 240.75    | 13.20 |
| 39  | 16             | 242.06    | 16.33              | 18 | 241.39               | 13.88 | 20 | 245.30    | 11.33 |
| 60  | 16             | 256.19    | 17.90              | 18 | 252.67               | 14.64 | 20 | 258.45    | 12.48 |
| 109 | 15             | 271.6     | 22.99              | 18 | 272.00               | 16.15 | 20 | 261.00    | 14.31 |
|     |                | Cortisone |                    |    | Cortisone            |       |    | Cortisone |       |
| 120 | 8              | 259.13    | 19.87              |    | 263.80               | 12.53 |    | 254.60    | 13.18 |
|     |                | Saline    |                    |    | Saline               |       |    | Saline    |       |
|     | 7              | 280.86    | 17.45              |    | 282.75               | 12.73 |    | 256.90    | 15.70 |

<sup>1</sup>In grams<sup>2</sup>Number of animals<sup>3</sup>Standard Deviation<sup>4</sup>Magnesium supplemented



TABLE VIII

Mean Number of Radioactive Disintegrations in the Root Tip of the  
Right Mandibular Incisor of Normal, Control<sup>1</sup> and Deficient Rats

| Group     | Time <sup>2</sup> | No. of<br>Root Tips | DPM <sup>3</sup> /Sample |                    | DPM/mg  |                      |
|-----------|-------------------|---------------------|--------------------------|--------------------|---------|----------------------|
|           |                   |                     | Mean                     | S. D. <sup>4</sup> | Mean    | S. D.                |
| Normal    |                   |                     |                          |                    |         |                      |
| Cortisone | 7 AM              | 4                   | 5464.75                  | 182.42             | 4261.25 | 650.25               |
| Saline    |                   | 4                   | 3836.25                  | 391.49             | 3443.25 | 320.24               |
| Cortisone | 1 PM              | 3                   | 4511.33                  | 447.94             | 4354.67 | 658.60               |
| Saline    |                   | 3                   | 4301.00                  | 191.50             | 4309.00 | 1093.87              |
| Control   |                   |                     |                          |                    |         |                      |
| Cortisone | 7 AM              | 5                   | 5960.40                  | 563.93             | 6735.40 | 1366.90              |
| Saline    |                   | 4                   | 5871.50                  | 592.11             | 5391.25 | 1194.22              |
| Cortisone | 1 PM              | 5                   | 5564.00                  | 681.35             | 6702.60 | 1257.02 <sup>5</sup> |
| Saline    |                   | 4                   | 4343.00                  | 289.79             | 5058.00 | 785.42               |
| Deficient |                   |                     |                          |                    |         |                      |
| Cortisone | 7 AM              | 5                   | 3216.00                  | 420.80             | 2867.00 | 465.09               |
| Saline    |                   | 5                   | 2522.40                  | 881.71             | 2478.80 | 731.59               |
| Cortisone | 1 PM              | 5                   | 3523.40                  | 1322.12            | 3298.60 | 1132.51              |
| Saline    |                   | 5                   | 2081.80                  | 1015.28            | 1999.60 | 718.16               |

<sup>1</sup>Magnesium supplemented

<sup>2</sup><sup>3</sup>H-thymidine administration

<sup>3</sup>Disintegrations per minute

<sup>4</sup>Standard deviation

<sup>5</sup>Significant at the 0.05 level. All other counts are not significant at the 0.10 or greater level of probability.

Plate 1

Figure 1. A normal rat showing thick fur, light pink ears and periorbital tissue. (x4).

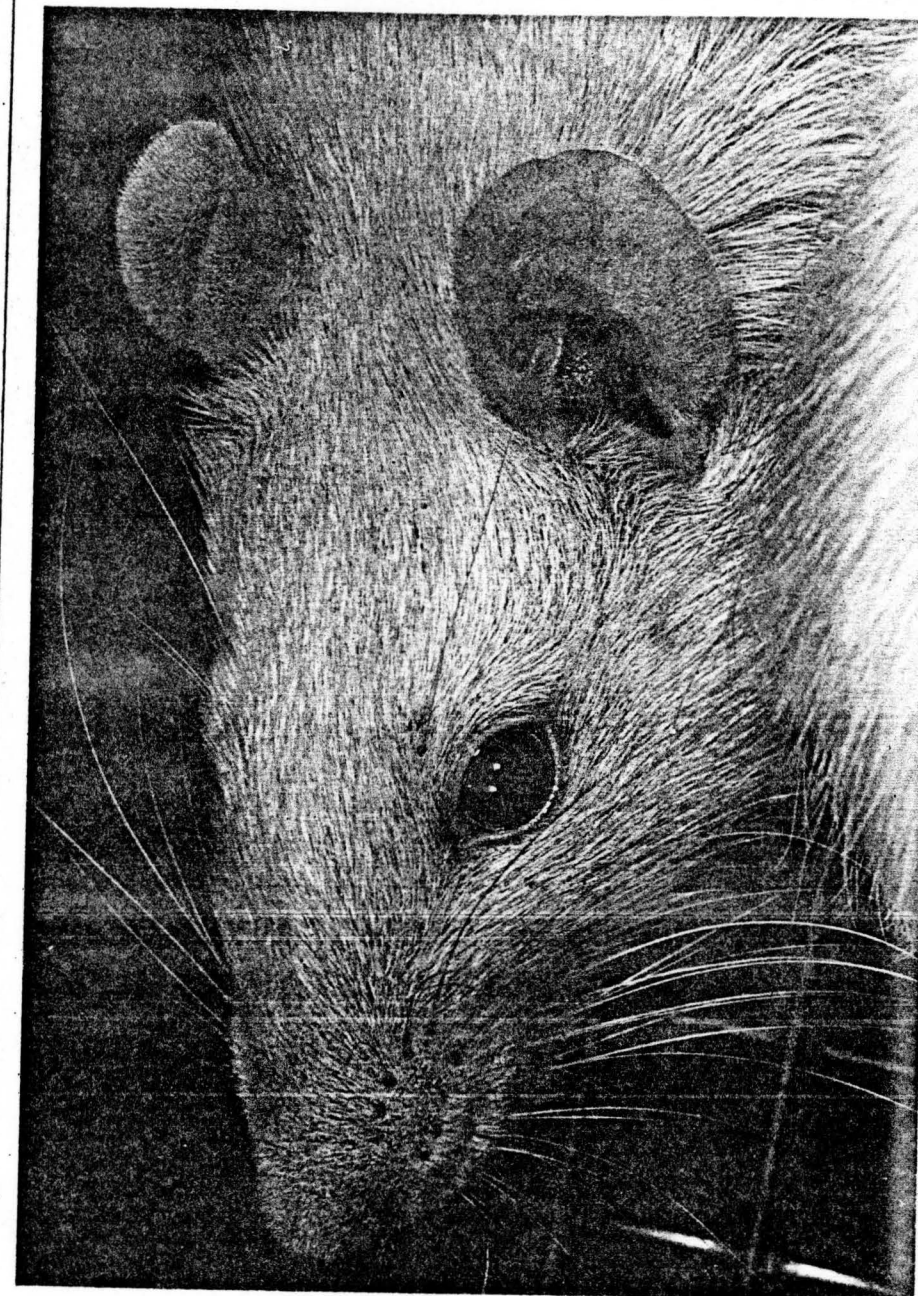


Figure 1

Plate 2

Figure 2. A normal rat with faint pink hind paw and tail, showing glossy, dense fur. (x4).

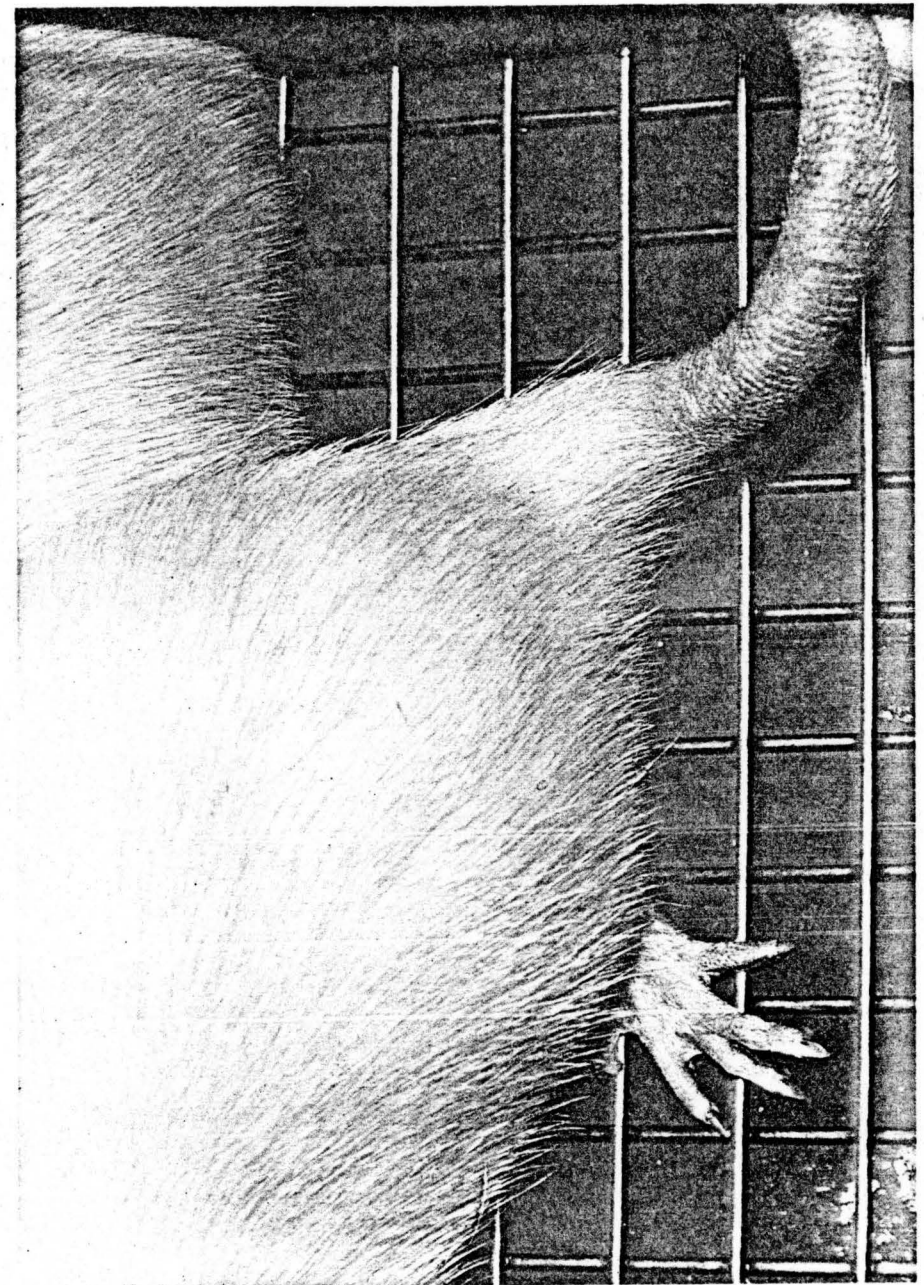


Figure 2



Figure 3. Magnesium deficient rat showing hyperemia of the right ear extending almost to the tip. Note two ulcerations on ear base. Compare with normal rat in Figure 1. (x4).

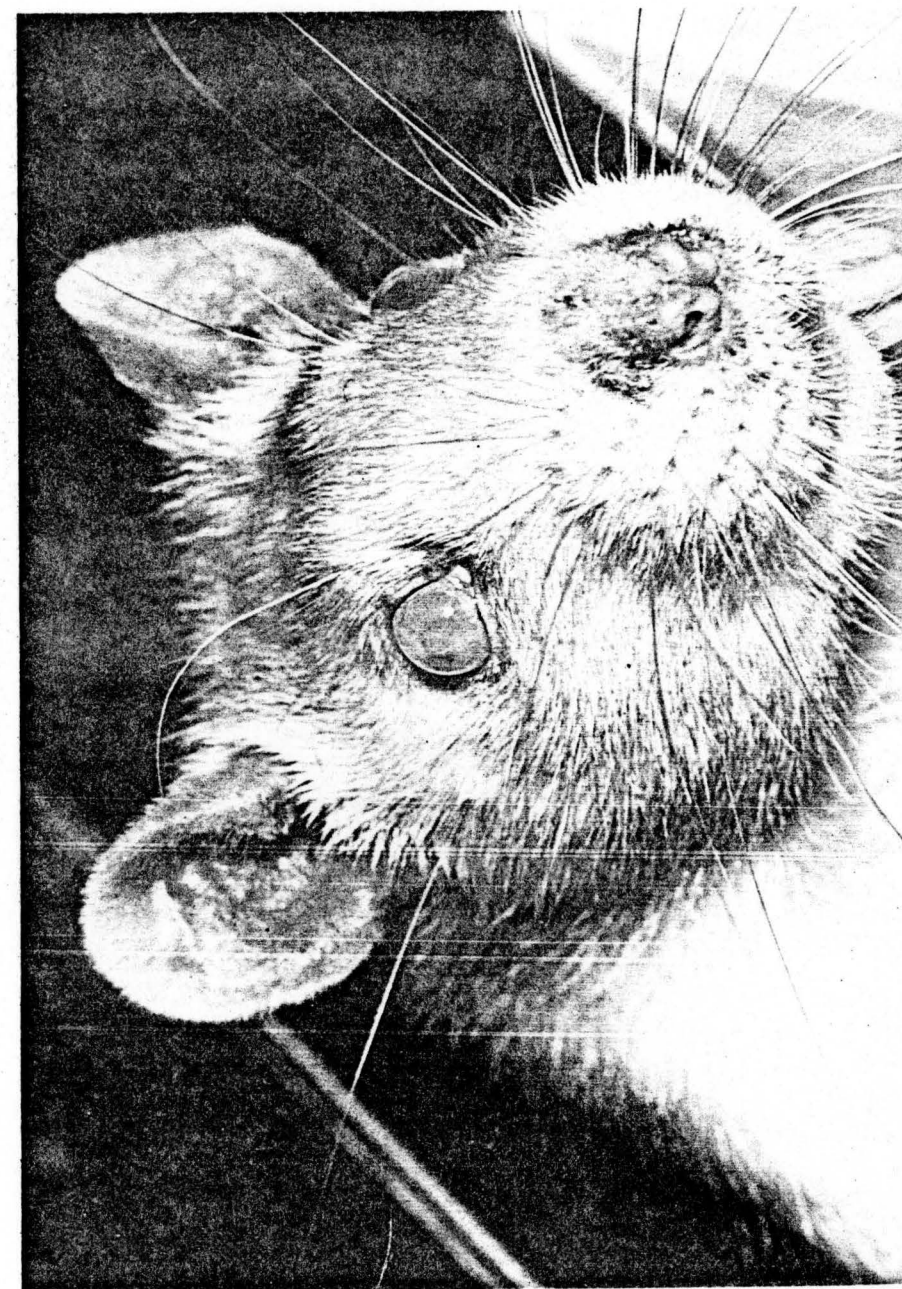


Figure 3

Plate 4

Figure 4. Inflammation and ulcerations of periorbital tissue in a magnesium deficient rat. Compare with normal rat in Figure 1. (x4).



Figure 4



Figure 5. Back and part of tail base of a magnesium deficient rat showing skin lesions and dull, brittle hair that is less dense than normal. Compare with normal rat in Figure 2. (x2).

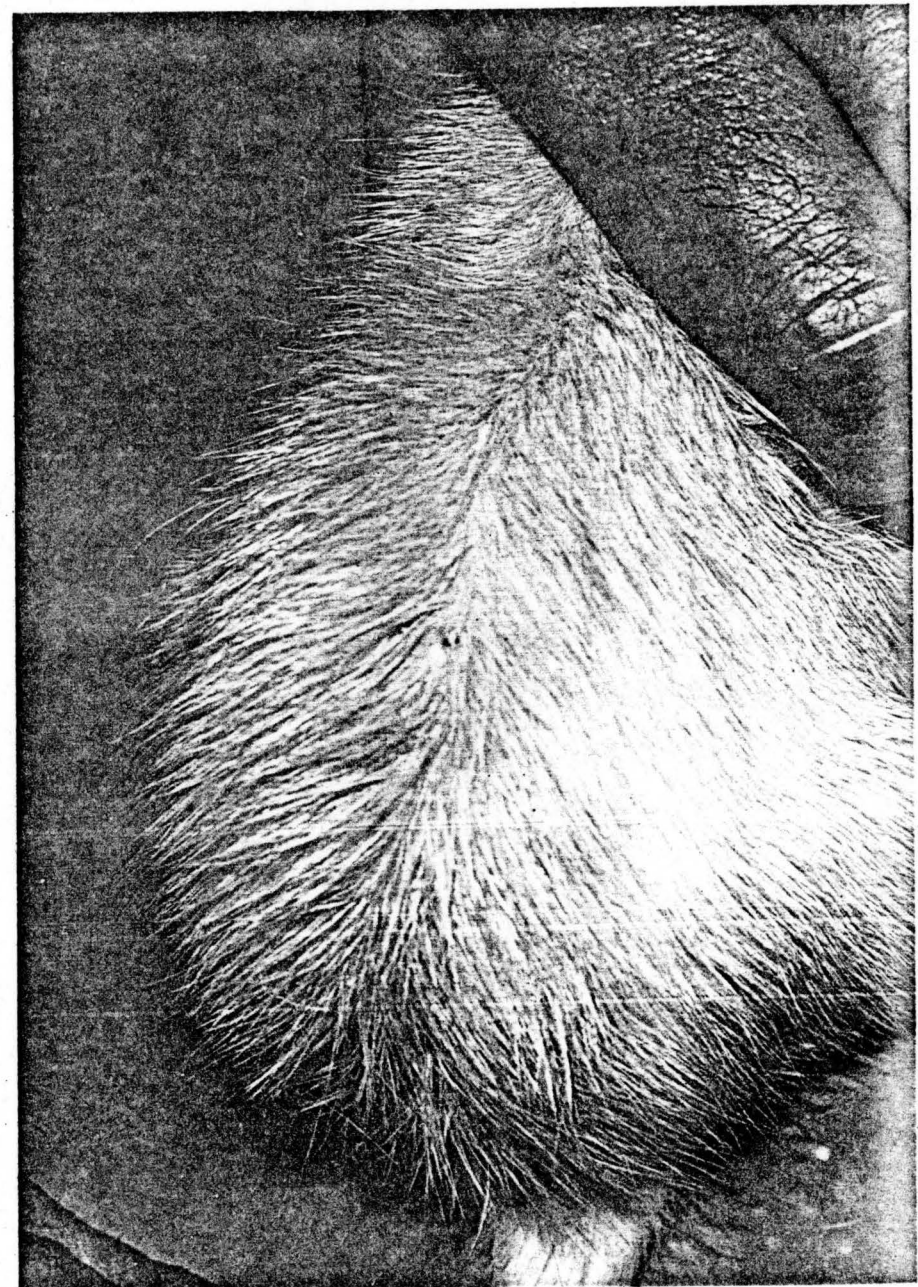


Figure 5

Figure 6. Normal rat incisors showing orange pigmentation and shiny surface. Note amount of hair on upper and lower lips. (x10).

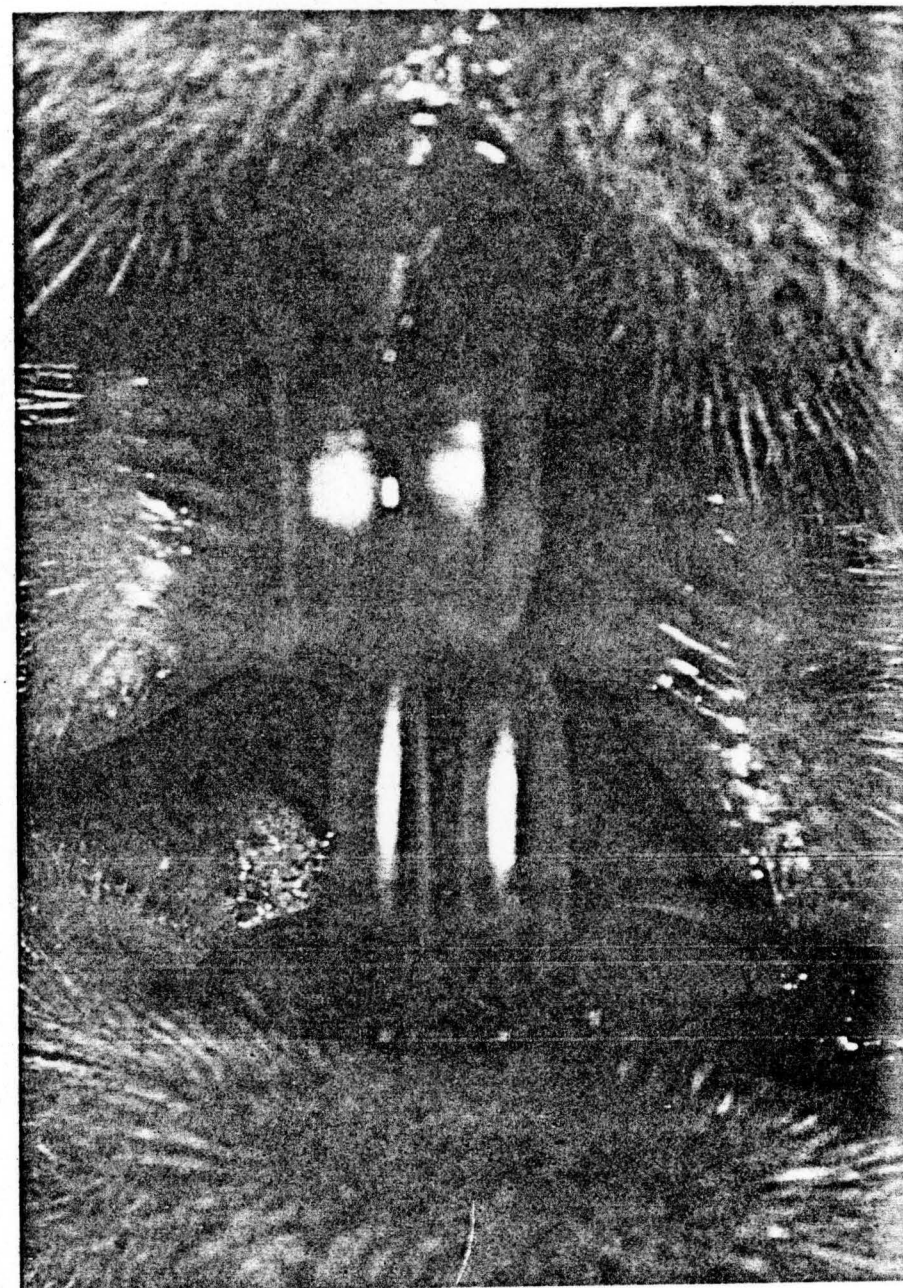


Figure 6



Figure 7. Magnesium deficient incisors showing some loss of pigmentation of the lower incisors and a dull finish. There is some hair loss on the lower lip. Compare with normals incisors in Figure 6. (x10).

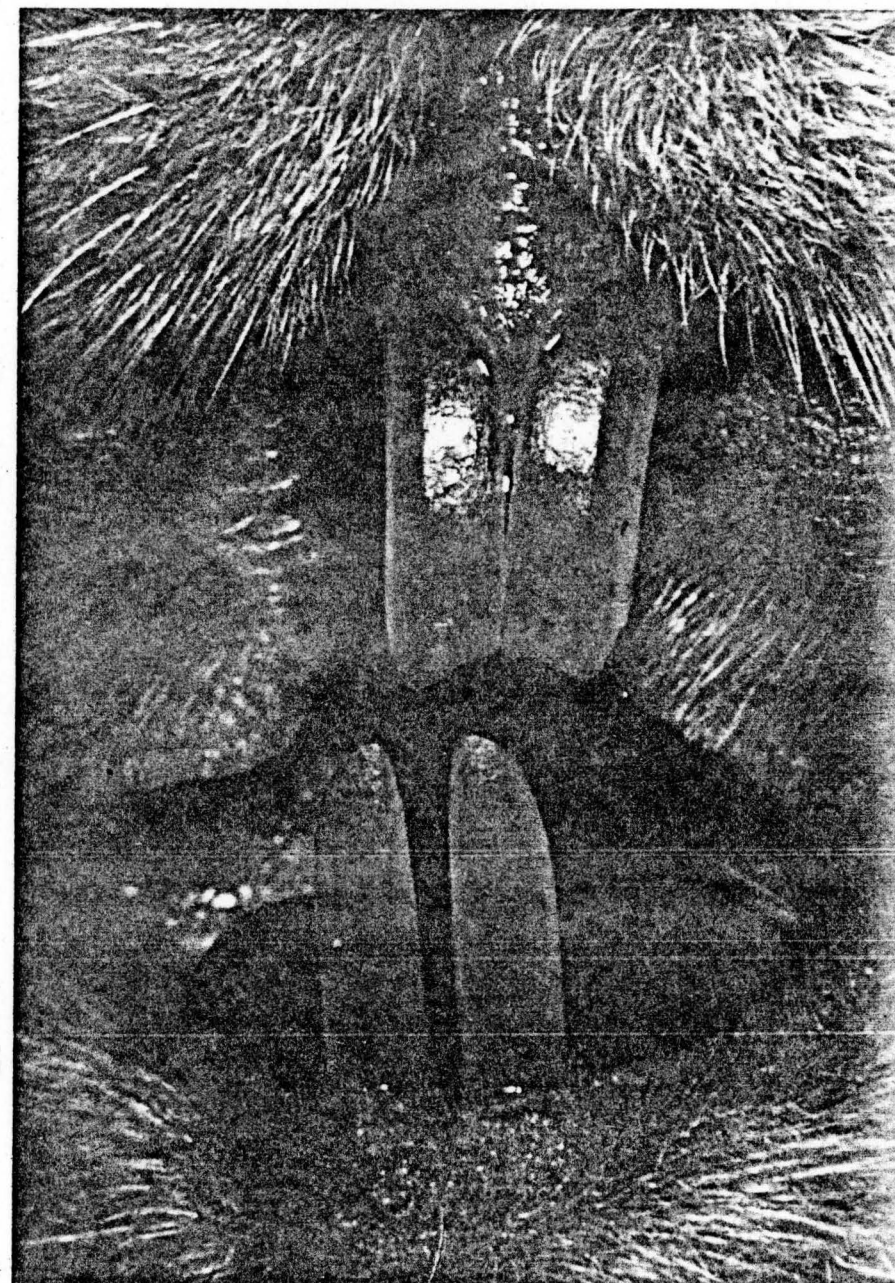


Figure 7



Figure 8. Incisors of a magnesium deficient rat showing dull, chipped maxillary incisors and white mandibular incisors, with only faint incisal pigmentation. Note excessive hair loss on lips. Compare with normal incisors in Figure 6. (x10).

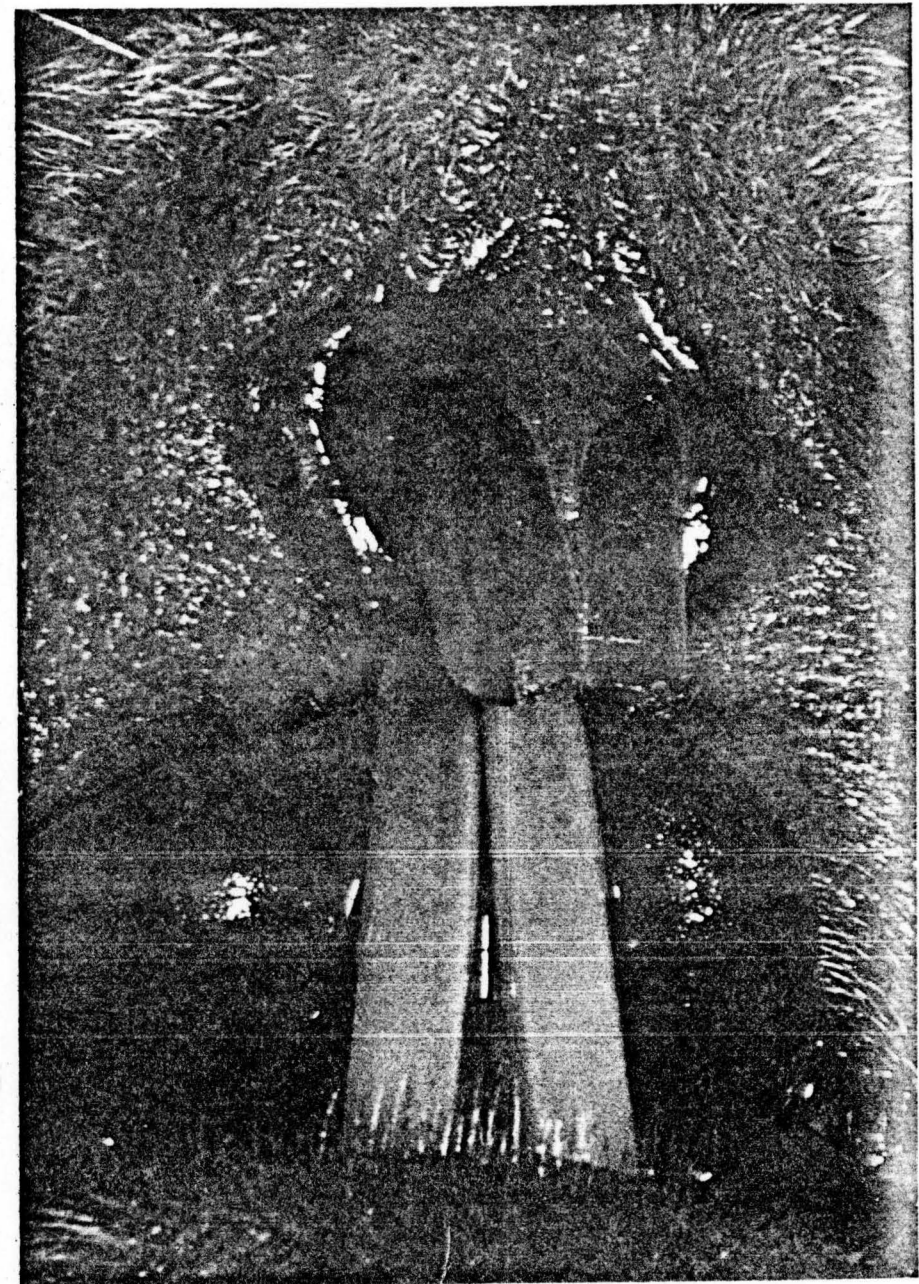


Figure 8

Plate 9

Figure 9. A midsagittal section of the normal labial (cervical) loop of a maxillary incisor showing the proliferative layers in which mitotic counts were made. Note the thin apical dentin. The enamel epithelium and odontoblast layers are also shown. (x93).

Figure 10. A high power photomicrograph of the four layers in which mitotic counts were made. (x421). Note the mitotic figures arrested at metaphase.

Abbreviations:

- MF - Mitotic Figures
- SI - Stratum Intermedium
- PA - Preameloblast Cell Layer
- PO - Preodontoblast Cell Layer
- P - Pulp

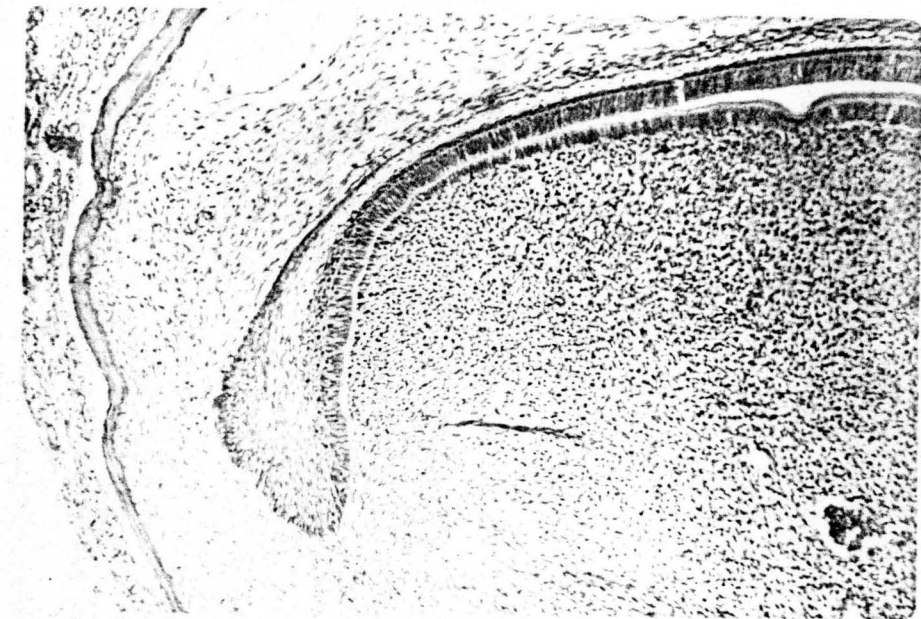


Figure 9

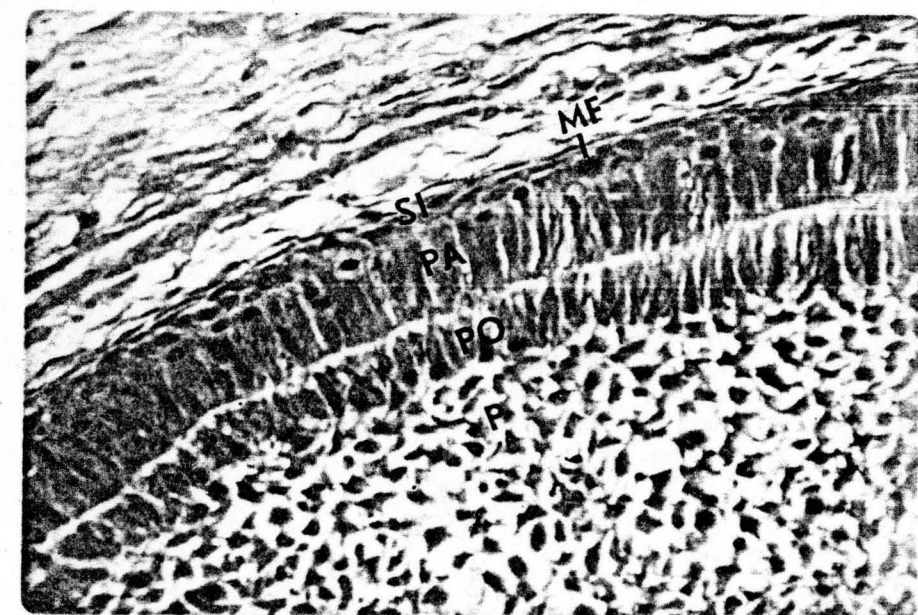


Figure 10



Plate 10

Figure 11. High power view, more incisally than Figure 10, showing deposition of dentin. (x421).

Abbreviations:

- SR - Stellate Reticulum
- SI - Stratum Intermedium
- A - Ameloblasts
- PR - Predentin
- O - Odontoblasts
- P - Pulp

Figure 12. Low power view of labial loop area of magnesium deficient incisor: Note excessive width of predentin and dentin as well as degenerated loop as compared to Figure 9. (x93).

Abbreviations:

- PR - Predentin
- D - Dentin
- L - Labial (cervical) Loop

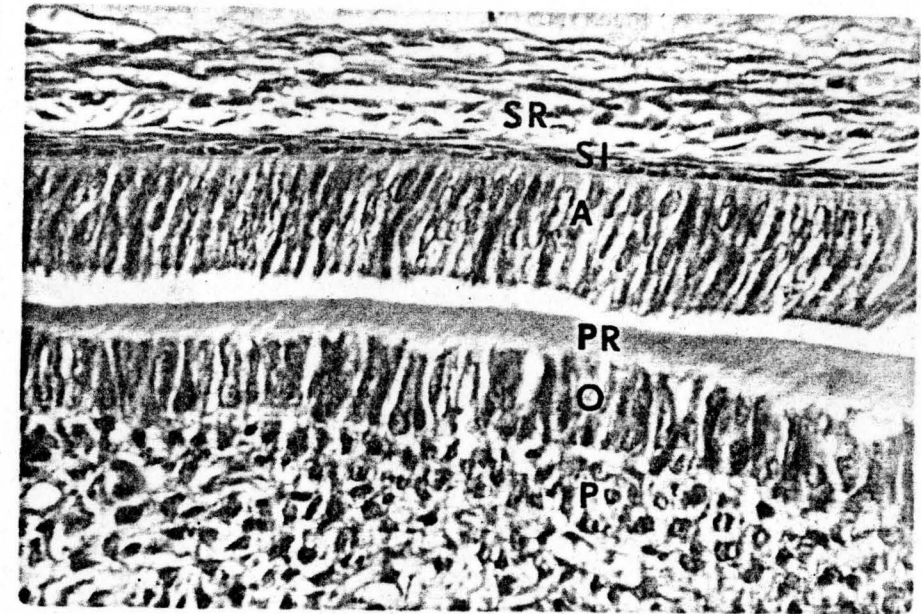


Figure 11

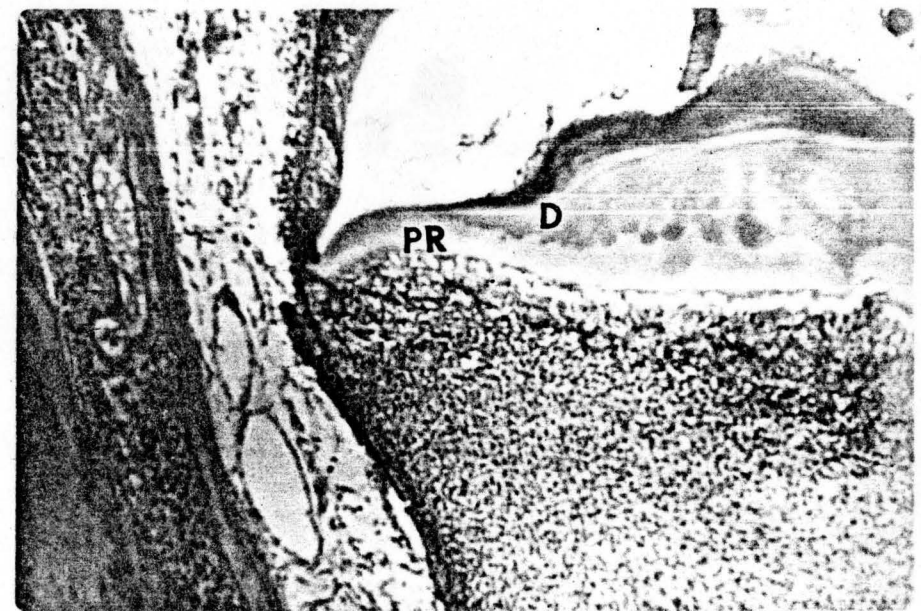


Figure 12

Plate 11

Figure 13. Lower power view of labial aspect of middle one-third of a normal incisor. Note that each layer is distinct and has a definite border. (x93).

Abbreviations:

- B - Alveolar Bone
- PL - Periodontium
- EE - Enamel Epithelium
- E - Enamel
- D - Dentin
- O - Odontoblasts
- P - Pulp

Figure 14. High power view of normal enamel and dentin. Note straight unbroken border between these layers. Also notice the rhythmic even manner in which the enamel and dentin tubules are arranged. (x421).

Abbreviations:

- E - Enamel
- D - Dentin

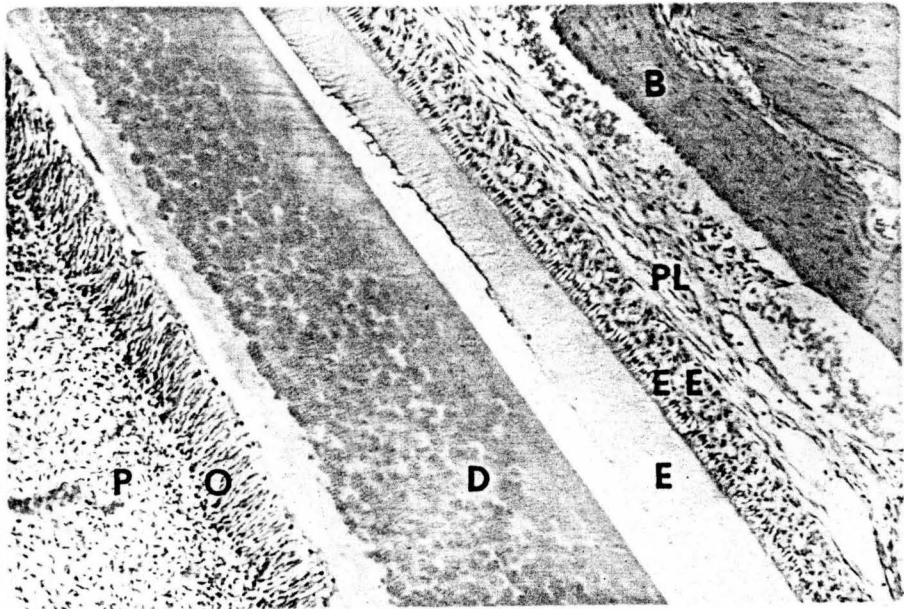


Figure 13

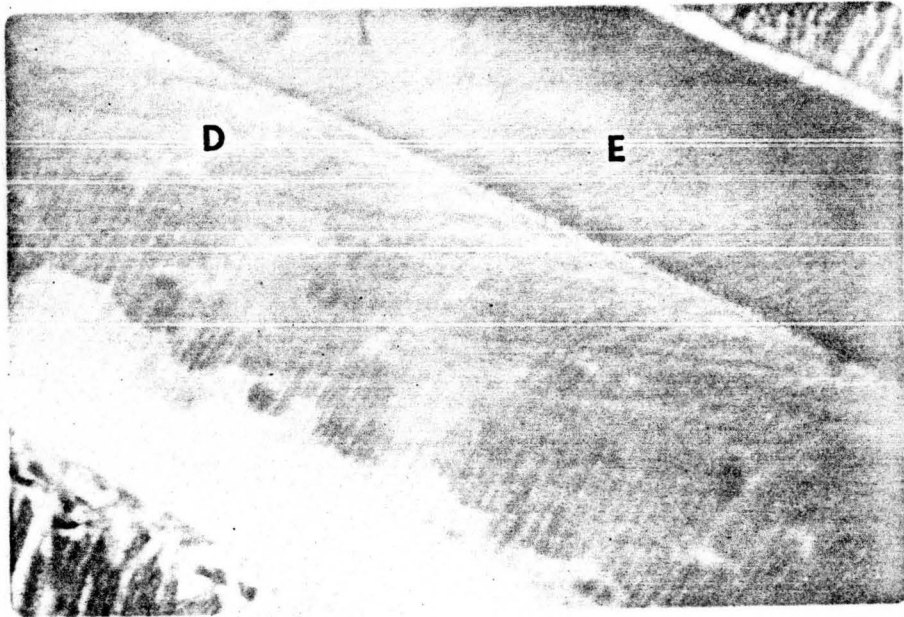


Figure 14



Figure 15. Low power of a magnesium deficient incisor (incisal one-third of tooth) showing stratified labial and lingual dentin. (x37).

Abbreviations:

- B - Knobby Alveolar Bone
- PL - Periodontal Ligament
- LD - Lingual Dentin
- P - Pulp
- PS - Fused Pulp Stones
- LAD - Labial Dentin

Figure 16. A higher power of figure 15 showing knobby alveolar bone undergrowing rapid resorption and a widened disorganized periodontium. (x93).

Abbreviations:

- B - Knobby Alveolar Bone
- PL - Periodontal Ligament
- LD - Lingual Dentin

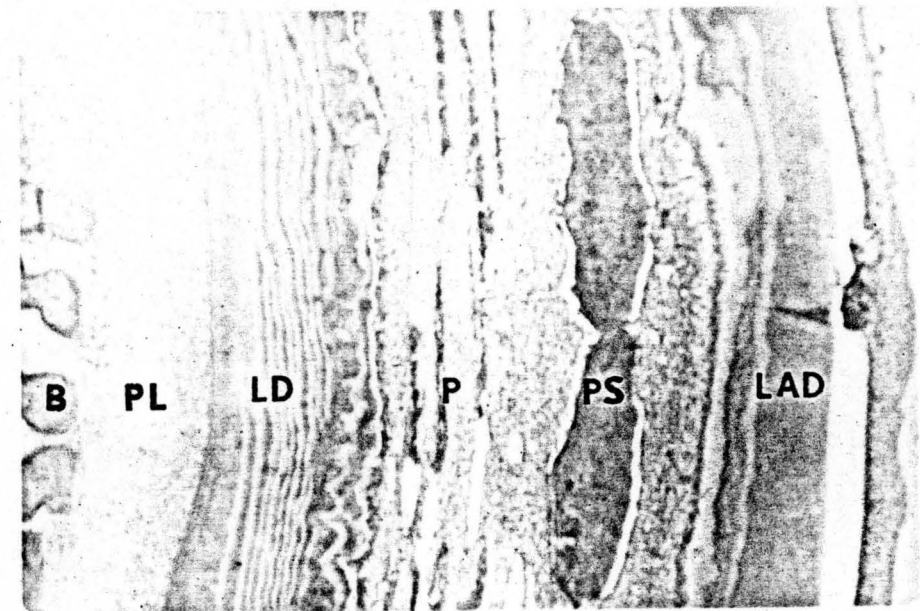


Figure 15

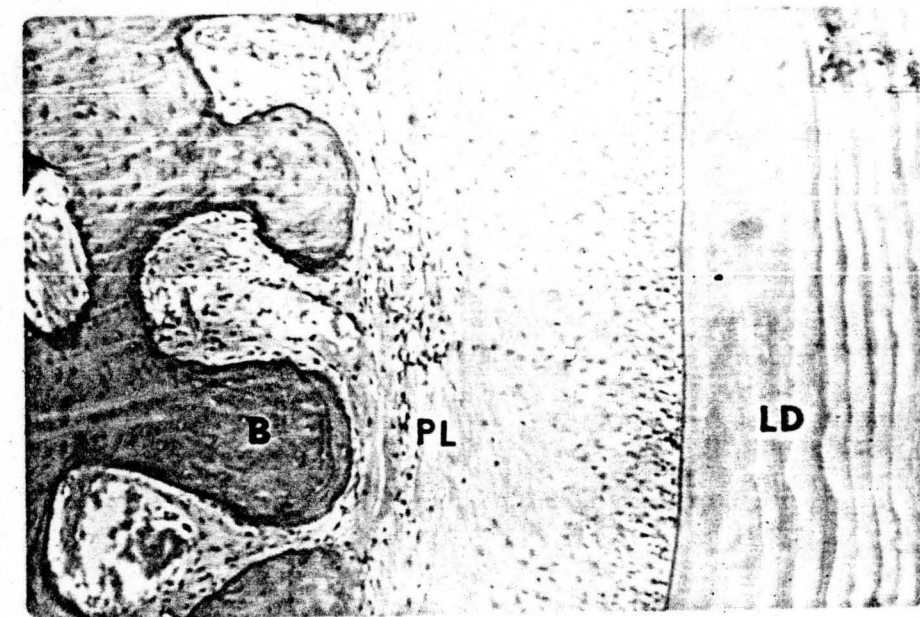


Figure 16

Plate 13

Figure 17. A high power view of middle one-third of normal incisor. (x421).

Abbreviations:

- A - Ameloblasts
- B - Papillary Layer
- PL - Periodontium
- B - Alveolar Bone

Figure 18. An atrophied enamel epithelium in a magnesium deficient incisor comparable to Figure 17. (x421).

Abbreviations:

- A - Ameloblasts
- P - Papillary Layer
- PL - Periodontium

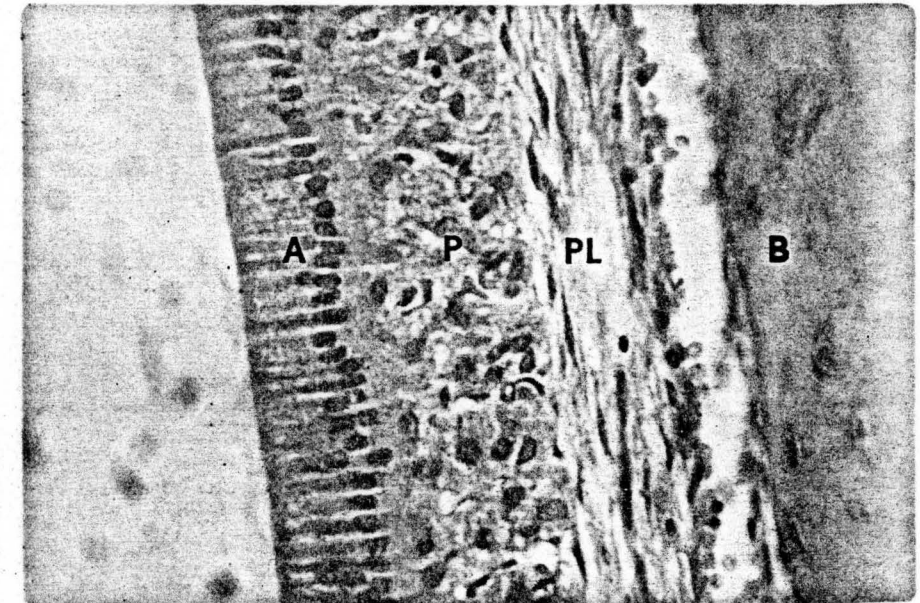


Figure 17

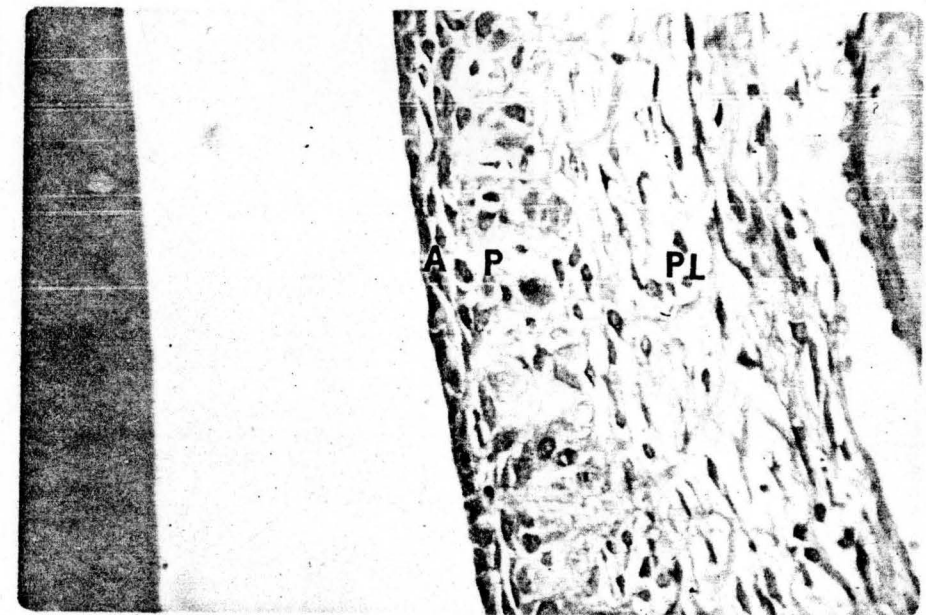


Figure 18



Figure 19. A magnesium deficient incisor showing calcific degeneration of a pulpal capillary, resembling dentin. Remnants of the capillary wall can still be seen. (x421).

Abbreviations:

P - Pulp

D - Denticle

C - Entrapped Capillary

Figure 20. A magnesium deficient incisor showing degenerated ameloblast with villus-like papillary layer still recognizable under high power. (x421).

Abbreviations:

E S - Enamel Space

E E - Enamel Epithelium

P L - Periodontium



Figure 19

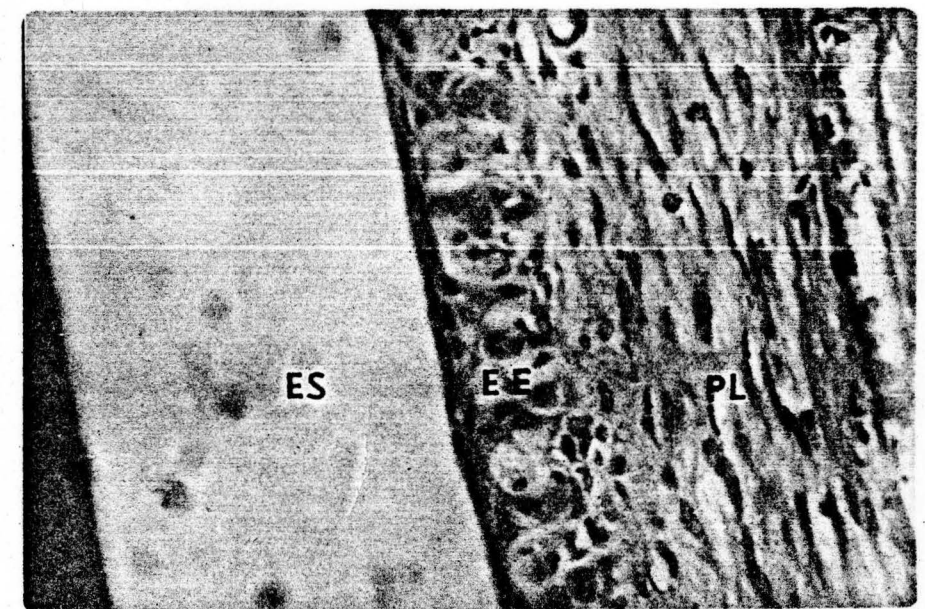


Figure 20

Plate 15

Figure 21. High power view of pulpal invagination into the dentin of a magnesium deficient incisor (incisal one-third). (x421).

Abbreviations:

D - Dentin Spur

P - Pulp

Figure 22. Photomicrograph of same incisor as shown in Figure 21; slightly more incisal. Note entrapped pulpal cells surrounded by stratified dentin. (x421).

Abbreviations:

P - Pulp Islands

S - Stratified Dentin

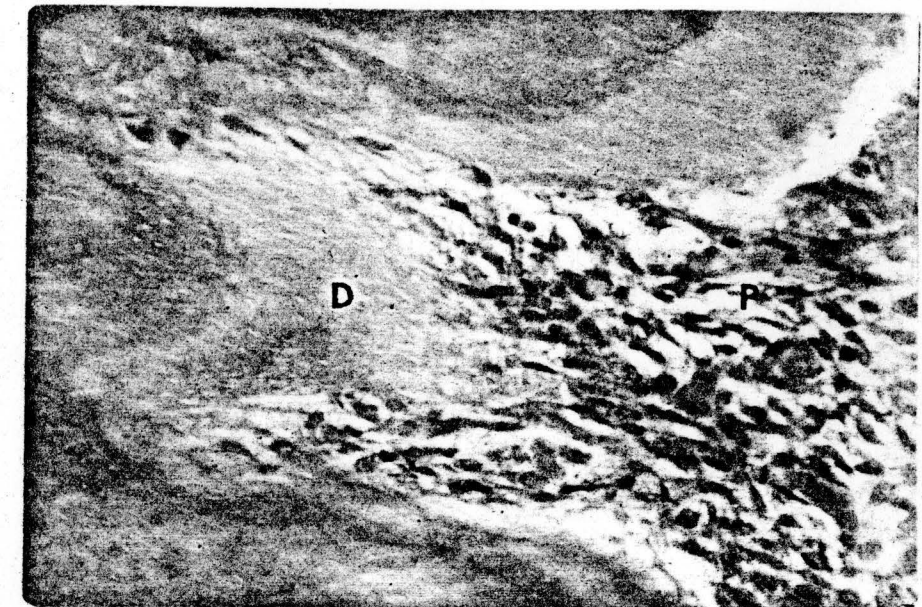


Figure 21

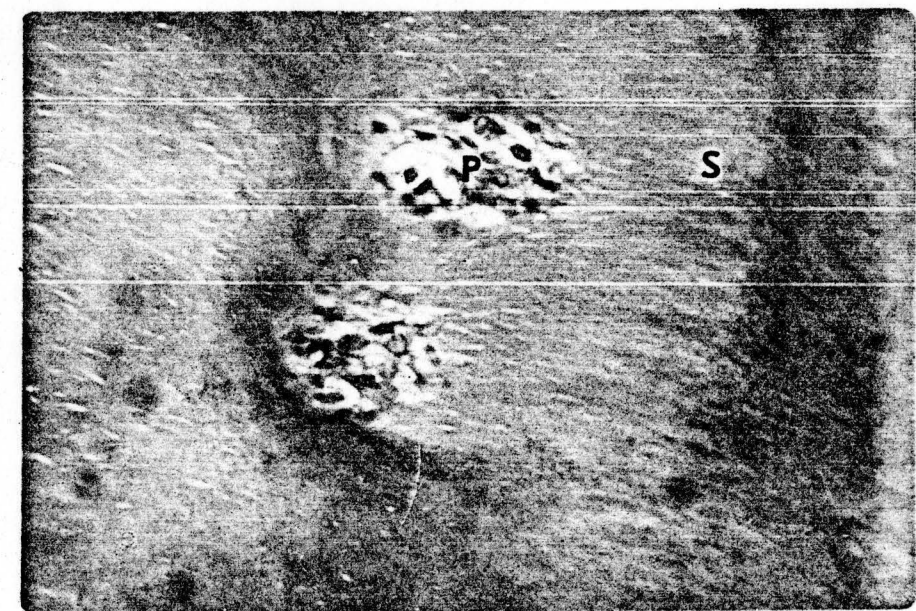


Figure 22



Figure 23. Atrophied tip of cervical loop in magnesium deficient incisor. Note scarcity of mitotic figures. (x421).

Figure 24. Atrophied cervical loop of magnesium deficient incisor. Note disorganized appearance and fibrous connective tissue invasion of stratum intermedium. (x421).

Abbreviations:

- S - Stratum Intermedium
- PA - Preameloblasts
- O - Odontoblasts
- P - Pulp



Figure 23

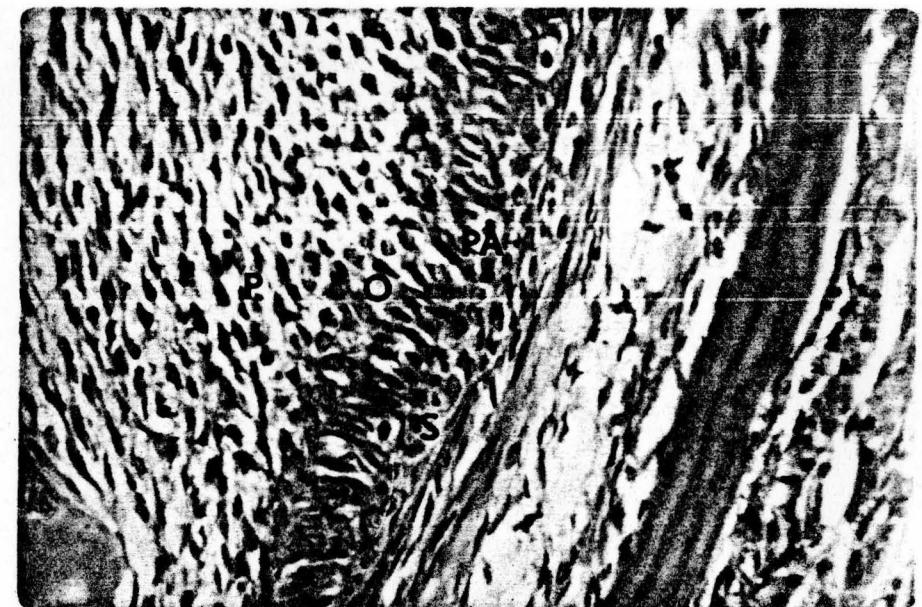


Figure 24

Figure 25. High power photograph of degenerated enamel epithelium. No ameloblasts or papillary layer are evident (x421).

Abbreviations:

E - Enamel Epithelium

D - Dentin

Figure 26. Lower power view of the basal area of an incisor of a magnesium deficient rat. The dentin is overgrown, folded and irregular. Note degenerated cervical loop at far left. Compare with Figures 9 and 12. (x93)

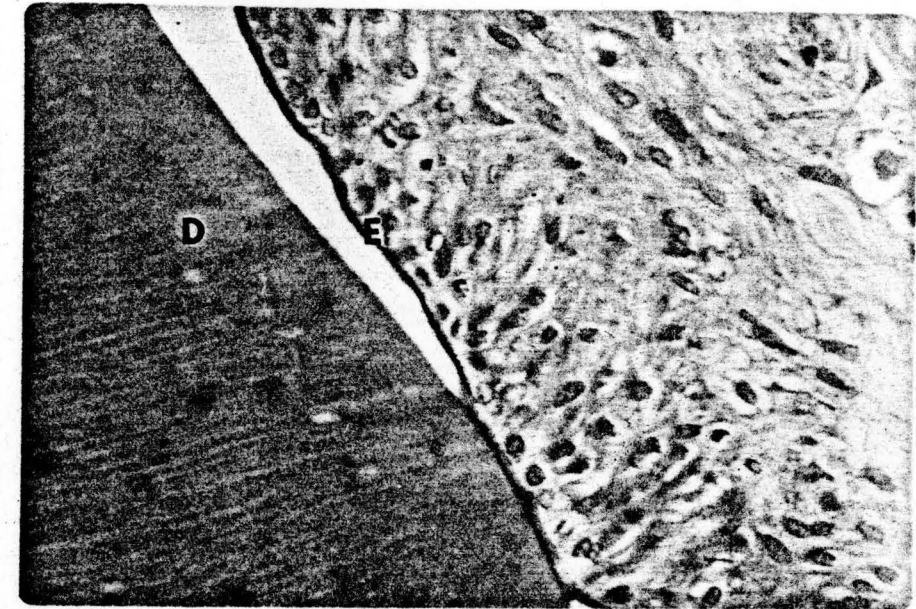


Figure 25



Figure 26



Plate 18

Figure 27. Low power view showing atrophied disorganized enamel epithelium. Note the epithelial pearls. Note cellular infiltration and severe breakdown of dentin. The area shown is at the middle one-third of a magnesium deficient incisor. (x93).

Abbreviations:

EE - Enamel Epithelium

P - Pearl

D - Dentin

E - Enamel

Figure 28. High power view of Figure 27. Note enamel pearl surrounded by disorganized and atrophied enamel epithelium. Note cuboidal shape of ameloblasts. (x421).

Abbreviations:

PA - Papillary Layer

A - Ameloblasts

P - Pearl

D - Dentin

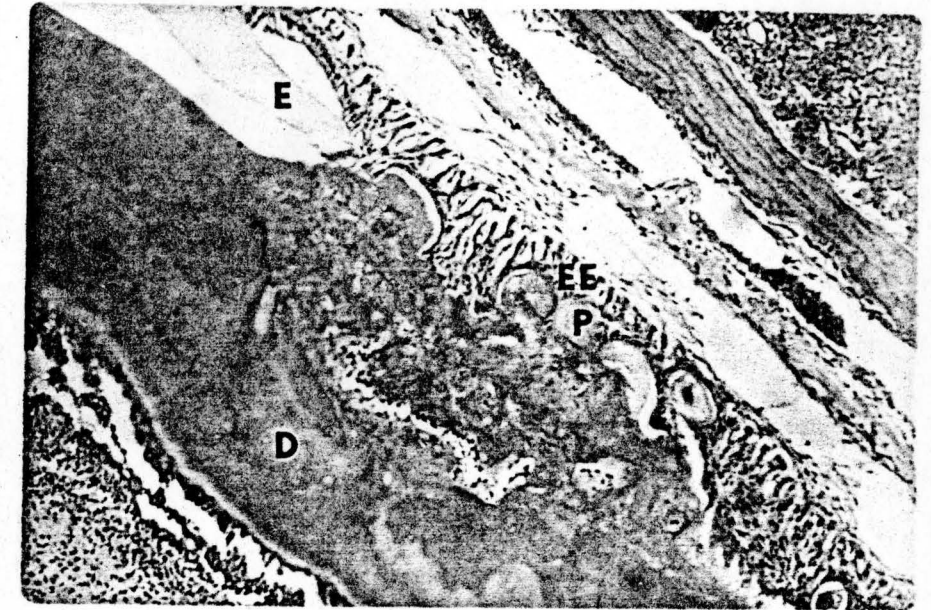


Figure 27



Figure 28

Plate 19

Figure 29. Low power view of magnesium deficient incisor (middle one-third) showing degenerate enamel epithelium and enamel matrix. Note two odontoblast layers. (x93).

Abbreviations:

- EE - Enamel Epithelium
- P - Pearl
- E - Enamel
- D - Dentin
- O - Odontoblast Layers

Figure 30. High power view of Figure 29 showing cellular invasion of enamel by epithelium, enamel destruction and apparent removal. (x421).

Abbreviations:

- EE - Enamel Epithelium
- ER - Enamel Removal
- E - Enamel

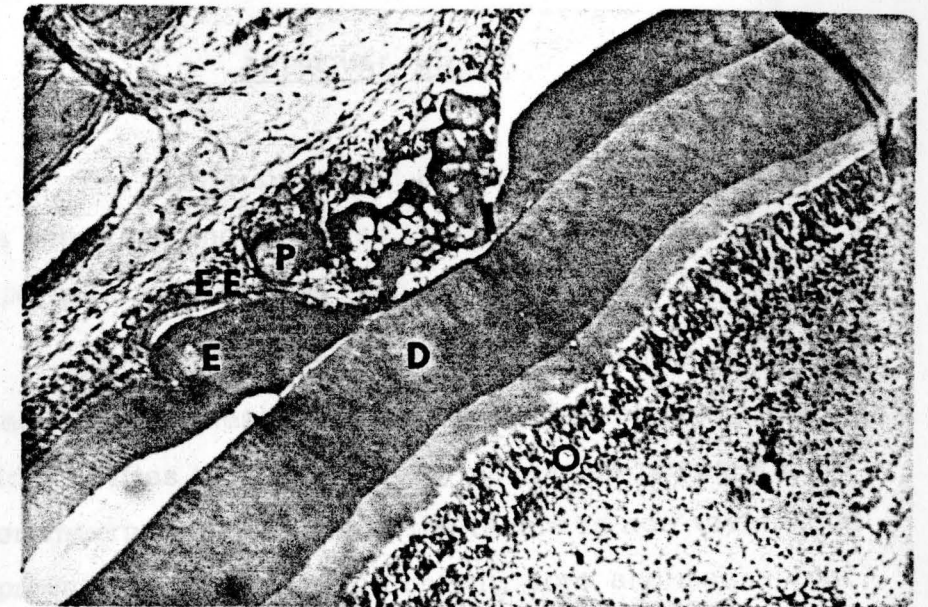


Figure 29

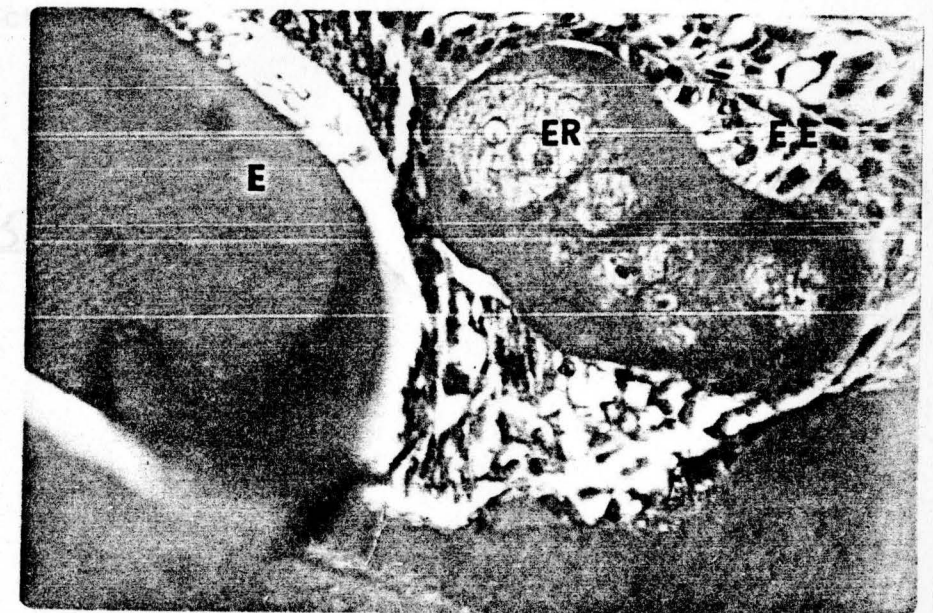


Figure 30

APPROVAL SHEET

The thesis submitted by Edward Stanley Salkin has been read and approved by three members of the Department of Oral Biology.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

5-18-73

Date

Michael L. Kieley Ph.D.

Signature of Advisor